A MORPHOLOGICAL AND GENETIC REVIEW OF THE *PARDOSA GROENLANDICA* SPECIES COMPLEX (ARANEAE: LYCOSIDAE)

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A

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Abstract

The *Pardosa groenlandica* species complex comprises seven recognized species, *P. groenlandica* (Thorell 1872), *P. dromaea* (Thorell 1877), *P. tristis* (Thorell 1877), *P. prosaica* Chamberlin and Ivie 1947, *P. bucklei* Kronestedt 1975, *P. albomaculata* Emerton 1885, and *P. lowriei* Kronestedt 1975. These species have overlapping distributions, creating sympatric occurrences with at least one other member of the complex. They can be found in Greenland, throughout Canada, and occur in the United States from the Rocky Mountains to the Pacific coast, through Alaska, and as far as eastern Siberia. These species' genitalia, which bear the primary diagnostic characters, are very similar and show large amounts of within-species and within-population variation. Because of this, they have seen various levels of taxonomic splitting and lumping from one species to the presently recognized seven. I evaluated the utility of the existing morphological diagnostic characters which, if geography is ignored, successfully diagnose only four species (*P. albomaculata, P. lowriei,* and *P. bucklei*, with the remaining species synonymized under *P. groenlandica*). Additionally, I sequenced five genes, two mitochondrial (COI & NDI), and three nuclear genes (ITS1, 5.8S, and ITS2) of 144 specimens, to help clarify the taxonomy of the species complex. All seven species showed some level of polyphyly or paraphyly in their gene trees. A population genetics analysis of *P. groenlandica* and *P. tristis* from Colorado populations failed to find molecular divergence between the populations, raising questions about *P. groenlandica* occurring in Colorado, and/or the validity of *P. tristis.* These results question the value of using this genetic dataset to test species delineated using traditional taxonomic methods in the

groenlandica species complex of the genus *Pardosa.* Reconciliation is likely only when genetic markers are studied that match the timing and rate of the observed phenotypic changes.

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Chapter 1

The designation and morphological review of the *Pardosa groenlandica* species complex

Abstract

Contained within the genus *Pardosa* the taxonomically confusing *Pardosa groenlandica* subgroup, designated as *P. groenlandica, P. dromaea, P. tristis, P. prosaica,* and *P. bucklei,* was combined with *P. albomaculata* and *P. lowriei* and here designated as the *Pardosa groenlandica* species complex. For clarification the taxonomic history of the species complex is covered, as are the various geographic and morphological means for species identification. In order to test the utility of the morphological identification information alone, a randomly assembled set of specimens were identified, keeping the geographic information hidden. Identifications made using only the morphological identification information resulted in difficulty in distinguishing *P. groenlandica, P. dromaea, P. tristis,* and *P. prosaica* from each other, with a 32% success rate (only slightly better than chance). Morphometric measurements similar to those made in the previous *groenlandica* subgroup designation, which included *P. groenlandica, P. dromaea, P. tristis, P. prosaica,* and *P. bucklei*, were replicated with freshly caught specimens, and similar results were obtained. Additionally, the genitalic morphology is discussed. The possible need to synonymize *P. dromaea, P. tristis,* and *P. prosaica* with *P. groenlandica* is discussed. The other species complex members were found to be identifiable using published morphological identification information. This review results in the species complex consisting of either the currently accepted seven

species, or four species if the synonymy of *P. dromaea, P. tristis,* and *P. prosaica* with *P. groenlandica* is accepted.

Introduction

Arthropods are the most diverse animal component of terrestrial ecosystems (Kremen *et al.* 1993), and the economic value of this diversity is calculated in the trillions of dollars (Costanza *et al.* 1997). Spiders are well known and important predators that have received considerable ecological attention (Wise 1993, Sundberg and Gunnarsson 1994, Rajeswaran *et al.* 2005, Hooks *et al.* 2006). However, our understanding of the evolutionary and ecological diversity of many spider groups is still rudimentary. One such group is the genus *Pardosa*, with over 550 species, 77 of which occur in North America. This genus is one of the most speciose members of the wolf spider family Lycosidae (Platnick 2011). *Pardosa* are small (3.0 mm-12.0 mm) spiders with a relatively high cephalic region and nearly vertical sides of the face (Vogel 2004). There are additional coloration and genital characters that define the 14 different species groups recognized by Vogel (2004).

The *Pardosa groenlandica* species complex, as designated here, comprises seven species that are distributed across the Northern Hemisphere from Greenland to eastern Asia, extending south to the Great Lakes region in the eastern United States and along the Rocky Mountains into New Mexico in the west (Figures 1.1 and 1.2). *Pardosa groenlandica* (Thorell 1872) is the most widely distributed member, being found from Greenland to the Yukon and south to Colorado along the Rockies, *Pardosa prosaica* Chamberlin and Ivie 1947 is limited to Alaska and eastern Asia; *Pardosa tristis* (Thorell 1877) occurs from the Rocky Mountains west to the Pacific

Ocean and North into British Columbia; *Pardosa dromaea* (Thorell 1877) occurs from the foothills of the Rockies east to the Mississippi River area; *Pardosa lowriei* Kronestedt 1975 occurs in the coastal mountains of British Columbia south to Oregon; *Pardosa albomaculata* Emerton 1885 has been collected in the higher mountains of New England, Northwest Territories, and Alaska, but likely occurs in-between; *Pardosa bucklei* Kronestedt 1975 occurs across the west from Colorado to Washington and north into Alberta and British Columbia.

This species complex is within the *modica* group (Dondale and Redner 1990, Vogel 2004) which contains 25 species found in North America. The *groenlandica* subgroup (comprising *P. groenlandica, P. tristis, P. dromaea, P. bucklei,* and *P. prosaica*) was recognized and revised by C. D. Dondale in 1999 using morphological data. The other two species (*P. lowriei* and *P. albomaculata*) were morphologically reviewed by Kronestedt (1975) and had either been considered variants of, or were once synonymized under, *P. groenlandica*. It is for this reason that I have chosen to unite the five species of Dondale's (1999) *groenlandica* subgroup with two additional species to form the *groenlandica* species complex to avoid confusion with Dondale's subgroup.

Even though *Pardosa* is one of the most speciose genera within the lycosids, the genus has been found monophyletic in higher-order molecular studies (Vink *et al.* 2002, Murphy *et al.* 2006), with species sampled from three continents. These studies provide molecular support that the morphological characters used to define *Pardosa* are homologous.

This first chapter reviews the history of the species complex and the means used for species identification. It then addresses whether the species can be reliably

identified using the previously published identification characters.

History of the *groenlandica* species complex members

The history of the species included in this complex is best understood if explained chronologically. The complex began with the description of *Pardosa groenlandica* by Tamerlan Thorell from a collection trip in 1871 to Greenland (Thorell 1872). A few years later, Thorell was sent a set of spiders from a trip into the western United States collected by A. S. Packard, collected in the Rocky Mountains in 1875. From this collection Thorell described three more members of the species complex, all from around the Denver, Colorado area: *Pardosa indagatrix* from Denver (later changed to *P. dromea* by Thorell in 1878 because the epithet *indagatrix* was preoccupied by an older name in *Lycosa*), *Pardosa iracunda* from Pike's Peak (later synonymized with *P. groenlandica* by Emerton in 1894), and *P. tristis* from Idaho Springs and Manitou Springs (Thorell 1877). It would appear that slight morphological differences were considered important for species-level determination by Thorell, as both *P. tristis* and *P. dromaea* were described from only a few female representatives (two specimens of *P. tristis* and one of *P. dromaea*), all described as being in poor shape by Thorell, and also appearing very similar to the previously described *P. groenlandica.* Additionally, *P. iracunda* was described from a single male and female pair from two different sites on Pike's Peak.

These few samples were too small for Thorell to understand these species' morphological variation. This was revealed in his description of *P. indagatrix*, in which he mentioned that this species may be a race of *P. groenlandica.* However, without specimens for comparison he felt it was safest to describe the potential

diversity and to name it as a new species. The type localities of the Colorado specimens do vary in elevation, with Denver at about 1600 m, Idaho Springs at 2200 m, and Pike's Peak at 3900 m. However, the type locations are all only about 70 km apart from each other. It is not clear if Thorell took this into consideration when describing these as different species.

The next member of the complex to be described was *P. albomaculata*, named by J. H. Emerton (1885), who described the species from a few spiders collected in the White Mountains of New England. Later, Emerton had an opportunity to examine a larger set of spiders with specimens collected from the Rocky Mountains, Washington, Labrador, as well as Thorell's type specimens from Colorado. Upon examination of these specimens, he synonymized *P. tristis, P. dromaea, P. iracunda,* and *P. albomaculata* with *P. groenlandica* (Emerton 1894). It would appear based on this decision that Emerton considered this group to be one morphologically variable species. Emerton also synonymized *P. sinistra* with *P. groenlandica,,* which was also described from Colorado by Thorell (1877). *Pardosa sinistra* was removed from Emerton's synonymy by Kronestedt (1981) because of considerable morphological differences and was not used in this study.

The next species to be described was *Pardosa nebraska* (Chamberlin and Ivie 1942) described from Nebraska, which has since been synonymized under *P. dromaea* by Dondale and Redner (1990). They mentioned its similarity to *P. groenlandica* but indicated some differences in the palps and epigyna but omitted the details. Following the description of *P. nebraska*, Chamberlin and Ivie (1947) described *P. prosaica* and *P. tristoides* from specimens from Alaska. The latter name was subsequently synonymized under *P. albomaculata* by Kronestedt (1975).

Chamberlin and Ivie (1947) state how both species differ only in epigynal characteristics from *P. groenlandica*, which they also found in Alaska. These later descriptions demonstrated how Chamberlin and Ivie's use of slight morphological differences for species-level determination contrasted with Emerton's philosophy of the group, a philosophy Chamberlin had agreed with earlier in his career. In his 1908 revision of the Lycosidae Chamberlin included *P. groenlandica*, accepting Emerton's 1894 synonymy, and may have even seen Thorell's types. It can be assumed that he was familiar with Thorell's work on the group as he cites them in his revision.

For the first half of the 1900s there was considerable confusion in the use of the proposed species. In works with specimens from the Rocky Mountains and the Eastern United States, *P. groenlandica* was generally used (Gertsch 1933, Comstock 1940, Levi 1951, Levi and Field 1954), whereas specimens from farther west were often identified as *P. tristis* (Lowrie and Gertsch 1955, Lowrie 1967, Schmoller 1970). Roewer (1955) mentioned *P. tristis* and re-established *P. sinistra* as a species; however, Roewer's work lacks any explanation for the taxonomic decisions he made. The removal of *P. sinistra* from synonymy with *P. groenlandica* was not explained until later by Kronestedt (1981), using newly evaluated morphological characters.

The first modern attempt to address the taxonomy of these species was made by Torbjörn Kronestedt (1975) who, upon examining a large amount of American *P. groenlandica* material, re-established *P. albomaculata* as a valid species. He also described *P. bucklei* and *P. lowriei* from specimens previously thought to be variants of *P. groenlandica*. Unfortunately, all of Thorell's type material is lost, so Kronestedt was unable to clear up the confusion around *P. groenlandica, P. dromaea, P. tristis,* and *P. iracunda*. He mentioned a large amount of variation in specimens he examined

from the Rocky Mountains, but that these differed considerably from specimens from Greenland and Labrador. He did not make any mention of *P. prosaica*'s placement. Kronestedt did establish some key morphological characters dealing with the conductor and terminal apophysis of the palp that have been used for defining the species since. He also mentioned the need for a *Pardosa glacialis/groenlandica* group, but he left the definition of the group for later work, which was never completed.

Continued progress on this group was made by Dondale and Redner (1990), who re-established *P. dromaea* based on specimens they collected outside of Denver. They also synonymized *P. nebraska* under *P. dromaea*. Their re-establishment of *P. dromaea* was based on it having a generally smaller size, different habitat (i.e., grasslands as opposed to streamside or talus cobble), and a failed attempt to mate grassland specimens with specimens collected at higher elevations. They also defined the *Pardosa modica* group, which includes all members of the *glacialis/groenlandica* group mentioned by Kronestedt (1975). Following this work, Dondale (1999) established the *groenlandica* subgroup based on the structure of the conductor and the shape of the retrolateral process of the terminal apophysis (RPTA). This subgroup has five species: *P. groenlandica, P. bucklei, P. dromaea, P. prosaica,* and *P. tristis,* with *P. iracunda* being synonymized under *P. groenlandica.* Dondale established neotypes for Thorell's specimens but mentioned that the habitats in which they were originally collected had been drastically altered. He was unable to find any *P. tristis* near Idaho Springs, forcing the movement of the neotype location 30 km away to Mt. Evans (4300 m), a habitat very similar to Pike's Peak (3900 m), the type location of *P. iracunda* (=*P. groenlandica*). Also, no *P. dromaea* could be located in Denver,

forcing the neotype's location to 16 km outside of Denver. These five subgroup species are diagnosable using the morphology of the palp in combination with their distribution as described in Dondale (1999). However, Dondale mentioned a great amount of morphological variation and that several of the species may be sympatric. The most recent work with the species included in this complex was by Vogel (2004) who reviewed United States *Pardosa* with an emphasis on identification. She used the general shape of the arc of the embolus and the shape of the palea as identification aids, but did not remark on relationships or subgroup members. She also mentioned the similarity between species and the reliance on geography for successful identification.

Because of the previous taxonomic status of *P. lowriei* and *P. albomaculata*, I have combined them with the *groenlandica* subgroup designated by Dondale (1999) and establish the *Pardosa groenlandica* species complex.

Methods

I used 182 fresh specimens for this study: 2 *P. albomaculata*, 5 *P. bucklei*, 8 *P. dromaea*, 67 *P. groenlandica*, 8 *P. lowriei*, 30 *P. prosaica*, 55 *P. tristis*, species complex members as well as 3 *P. ourayensis* Gertsch 1933, and 4 *P. xerampelina* (Keyserling 1877) as outgroup species. These were largely collected during several collection trips I made from May through August 2009 in western North America (Figure 1.3). Outgroup species selected represent a within-*modica* group member *P. ourayensis,* and a non-*modica* group member *P. xerampelina.* Specimens were collected in 100% ethanol and stored at -20°C until identifications could be made. Identified specimens were sorted and databased in the University of Alaska Museum

Insect Collection, then stored at -80°C until extractions could be completed. Extractions and specimens used for this study have been deposited in the University of Alaska Museum (UAM) Insect Collection except for several specimens provided on loan by R. J. Adams (pers. coll.), Susan Wise-Eagle (pers. coll.), Gerry Blagoev (University of Ontario, Guelph), and Buzz Morrison (DMNS; Appendix A).

To ensure correct identification of new specimens, a voucher set of specimens used in Dondale's revision (Dondale 1999) was provided by Charles Dondale via the Canadian National Collection (CNC): 15 *P. groenlandica,* 8 *P. dromaea*, 10 *P. bucklei*, 9 *P. tristis,* and 16 *P. prosaica.* Scanning electron micrographs were taken using an ISI-SR50 microscope of these CNC voucher specimens for aid in identification. Additionally, specimens from the Denver Museum of Nature and Science (DMNS) arachnid collection were examined. These included specimens identified by B. Vogel, C. Dondale, T. Kronestedt, D. J. Buckle, W. Gertsch, H. K. Wallace, and me. Specifically, attention was paid to the characters used in the original descriptions and in more recent taxonomic works by Kronestedt (1975), Dondale and Redner (1990), Dondale (1999), and Vogel (2004).

To evaluate the consistency of the published characters for identification, newly collected specimens of *P. dromaea, P. tristis, P. groenlandica*, and *P. prosaica* were chosen that could be positively identified based on geography (from regions lacking sympatry with other species group members) and habitat alone. Of these, 58 specimens were randomly chosen for a blind identification analysis in which attempts were made to identify them using only the published morphological characters in Dondale (1999) and Vogel (2004), keeping the location and habitat information hidden. The percentage of correct identifications was calculated and notes on the

identifications were made.

The specimen set used for the blind identification was expanded to include an additional 23 specimens (9 *P. dromaea*, 10 *P. bucklei*, and 4 *P. prosaica*) from the CNC voucher set to replicate the morphometric analysis in Dondale (1999). These measurements included carapace width, carapace length, and the p/q ratio (Figure 1.4) of the epigyna, in which p is the length from the anterior end of the MS to the atrial sclerite, and q is the total length of the MS (Dondale 1999). These new data were compared to Dondale's results using a student's *t*-test for the average of each character. The differences in the means of new data for these characters were compared using an ANOVA.

Results

Using previously published identification characters, four species of the *groenlandica* complex species could be reliably identified. Both *P. albomaculata* and *P. lowriei* could by identified by the distinctive shape of their conductors (Figure 1.5) and epigyna (Figure 1.4), particularly the shape of the atrium, atrial sclerites, and medium septum (MS; Kronestedt 1975). The other species all share the distinctive flat conductor tip identified by Dondale (1999) as a character of the *groenlandica* subgroup. *Pardosa bucklei* could be correctly identified using the distinctive thick embolus and the shape of the MS, atrial sclerites, and atria (Figure 1.6). Additionally, *P. bucklei* has a significantly smaller epigyna ($P<0.0001$; d.f.=4, 42; F=10.37), ranging from 0.60 - 0.73 mm in length compared to 0.82 - 1.08 mm for all other species. This species showed little genitalic variation in specimens examined across its range. Additionally, *P. bucklei* is a smaller species and is generally found in a

grassy or debris-filled habitat adjacent to water (Dondale 1999, J. Slowik pers. obs., B. Vogel pers. obs.), whereas other *groenlandica* species complex members prefer large scree or cobble areas (Dondale 1999, Vogel 2004, Dondale pers. comm., Vogel pers. comm., J. Slowik pers. obs.).

The other four members of the species complex, *P. groenlandica, P. tristis, P. prosaica,* and *P. dromaea,* were found to lack distinctive morphological characters for reliable identification. Results of my blind identifications involving these four species resulted in 43% (25 of 58 specimens) being incorrectly identified using the published morphological characters alone (Dondale 1999, Vogel 2004). Because multiple characters are used for identification, 24% (14 of 58 specimens) were found to possess characters from two or three possible species. These specimens could not be identified confidently to a single species. However, of the potential species that these specimens could be, one of them had to be the correct species. Thus, if a determination had to be made, there was a 33% - 50% possibility that it would have been correct. The remaining 32% (19 of 58 specimens) were correctly identified based on the published characters for identification without the use of geographic or habitat information.

In particular, the shape of the retrolateral process of the terminal apophysis (RPTA; Figure 1.7), which was mentioned as a useful character for species identification of the subgroup by Dondale (1999), was found to be difficult to use and it produced inconsistent results. To observe the RPTA, dissection of the palp is required, and damage to the RPTA may occur. Additionally, a lot of variation was seen in the RPTA shape within males from a single population (Mt. Evans, CO). This could be due to two species in sympatry (*P. tristis* and *P. groenlandica*), as mentioned

as a possibility by Dondale (1999), or one polymorphic species. Additionally, an RPTA shape (Figure 1.7, *P. tristis*) not mentioned in Dondale was found in several specimens of *P. tristis* from Kamloops and Prince George, British Columbia, and one specimen from Mt. Evans, Colorado. The RPTA shape does not appear to conform to clearly distinct shape categories, but rather appears variable within populations and species.

The shape of the MS and size of the atria were found to be major characteristics used for identification. Three geographic variants were identified based on the shape of the MS, and represented the 32% of correct identifications in the blind trial. These are: an inverted T shape, a bottle shape, and an urn shape with a constricted anterior neck (Figure 1.8). The inverted T-shape MS variants are found west of the Rockies from New Mexico north to British Columbia in the Great Basin, and described as *P. tristis* by Dondale (1999). They may be characterized by a long, narrow MS abruptly widening in the posterior region, with the posterior edge often being almost flat. The urn-shaped MS variants were found in Alaska extending east into the Yukon, and were described as *P. prosaica* by Dondale (1999). This variant may be characterized by a narrow anterior region widening into a curved arc along the lateral edge, with a curved posterior edge. The size of the posterior MS expansion was variable. Male specimens from Alaska and Yukon were also found to have a constriction on the interior edge of the embolus (Figure 1.8). However, females collected in eastern Yukon and northern British Columbia with urn-shaped median septa were collected with males that did not have the constricted embolus. It would appear that the distribution of urn-shaped median septa extends beyond that of the constricted embolus. The bottle-shaped MS variant was found in specimens from

Newfoundland and Greenland and was described as *P. groenlandica* by Dondale (1999). This form is characterized by a relatively wide anterior region, widening somewhat then extending posteriorly and creating almost parallel lateral edges of the MS. These geographic variants were often collected from populations which also had individuals that had MS shapes not fitting one of the three previously described shapes.

A fourth shape, the "A" shape, was described as being found in both *P. tristis* and *P. prosaica* by Dondale (1999; Figure 1.9). This shape was found not to have a distinct distribution trend and was one source of error in five of the identifications. Misinterpretation of the urn shape resulted in the incorrect identification of eleven specimens due largely to the constriction of the anterior MS region (Table 1.1).

The majority of specimens had MS shapes that were a conglomeration of the four described shapes and did not present geographic patterns tied to MS morphology. Figure 1.9 b is an example, with a narrow anterior region widening similar to a bottle-shaped MS, a gradual curving lateral edge similar to an urn-shaped MS, and a flat posterior edge similar to an "A" or inverted T-shaped MS. Other specimens had a narrow MS that failed to expand posteriorly at all (Somers Beach, Montana, n=2). Specimens expressing median septa outside of the *sensu stricto* descriptions were often collected syntopically with specimens that did present one of the four described shapes, demonstrating considerable within-population variation in this character.

Comparison of the morphometric data with those of Dondale (1999) was only significantly different for one character, male *P. bucklei* carapace length (Figure 1.10, P=0.0039; d.f.=8; t=4.01,). My results showed *P. bucklei* to be significantly different from all other species included in the *groenlandica* subgroup for all measured

characters (Table 1.2, $P < 0.05$). *Pardosa dromaea* was found to be significantly smaller for all characters except for female carapace length ($P<0.05$). These data are in general agreement with Dondale's results and led to the same general conclusions, that *P. bucklei* and *P. dromaea* are smaller species.

Published species descriptions enabled the identification of four species, *P. albomaculata, P. lowriei, P. bucklei* and *P. groenlandica,* with the latter as an amalgam of *P. groenlandica, P. tristis, P. prosaica,* and *P. dromaea.*

Discussion

This review of the *groenlandica* species complex replicated and re-evaluated previous studies and results presented in previous taxonomic literature (Kronestedt 1975, Dondale and Redner 1990, Dondale 1999). Here I differ from Dondale's interpretation in concluding that the morphological variation present in *P. groenlandica, P. dromaea, P. tristis,* and *P. prosaica* is insufficient to warrant species designation. There are not clear morphological species boundaries; rather, there are morphological geographic trends. I found large amounts of genitalic variation in presumably conspecific members collected simultaneously (i.e., in the same place and time). Those who have worked with members of this subgroup and other *Pardosa* species groups have remarked on the large amount of genitalic variation among species and within populations (Holm 1939, 1967; Kronestedt 1975, 1986, 1988, 1993; Vogel 2004). I did not find any previously unused characters that might help to diagnose the named species. These results do not rule out the presence of multiple species living in sympatry, but they highlight the fact that if this is happening there is no consistent morphological way to separate the species.

This difficulty in defining species boundaries is not unexpected, as lycosid spiders, and particularly pardosine spiders, show a great deal of conservation in some genital structures across genera, while also showing large amounts of genital variation in other structures among populations and species (Wallace 1942, Dondale and Redner 1990). Additionally, spiders are thought to be prime candidates for evolutionarily fast reproductive morphology changes (Eberhard and Huber 2010). Pardosine spiders are also generalist predators, often being found over wide distributions and in differing habitats (Dondale and Redner 1990, Vogel 2004). Add to these factors a lycosid spider's ability to distribute via ballooning when young (Greenstone *et al.* 1987, Crawford *et al.* 1995), and the inference of species boundaries can be difficult.

These data also show that identification of *P. groenlandica, P. dromaea, P. tristis,* and *P. prosaica* based on morphology alone is not possible. Dondale (1999) provides *sensu stricto* descriptions which will identify some individuals of a population. For example, *P. prosaica* can be characterized by the urn-shaped MS and by the constriction on the embolus, *Pardosa tristis* by the inverted T-shaped MS, and *P. groenlandica* by the bottle-shaped MS. Additionally, specimens identified as *P. dromaea* were significantly smaller and showed a significantly smaller epigynal *p/q* ratio; however, there are no male attributes for identification of these last three species. Also, the differences in the morphological measurements that identify *P. dromaea* may be an artifact of the habitat, as the growth and development of juvenile *Pardosa* are affected by nutrition (Miyashita 1968, West-Eberhard 2005). The larger issue is that none of these *sensu stricto* forms exist in isolation from other variants showing a conglomeration of characters. This leaves the identification of the variants

to reliant on their proximity to specimens which are identified as one of the four species *sensu stricto* Dondale (1999).

If these four species are examined as a metapopulation, they appear to have morphologically distinct geographic races. Previous descriptions of these four species mention specific distribution or habitat areas as an aid in identification (Dondale 1999, Vogel 2004). Specimens from each of the geographic races could be viewed as a distinct species, thus the *sensu stricto* forms, without knowledge of morphologic variation present in the populations. Unfortunately, these morphological trends raise questions that morphology alone cannot answer. For example, the inverted T-shaped MS is only found in populations west of the Rocky Mountains. Other MS shapes are also found within these same populations. Therefore, it could be hypothesized that these are two distinct species in sympatry, or that the inverted T-shaped MS race is a historic geographic race that is experiencing some introgression from a more variable shaped MS race representing MS shapes other then the inverted T-shaped. If the latter hypothesis is assumed, it questions these spiders' ability to distribute young long distances via ballooning, as no specimens with an inverted T-shaped median septa were found north or east of the Rockies.

The existence of these geographic races inevitably raises the issue of subspecies. Subspecies are rarely used in spider taxonomy. In the few cases in which they have been employed, they define populations showing some geographic morphological trend, in which the extent of mixing of the different races is unknown (Schick 1965, Dondale 1967). Subspecific designation is also rarely used in cases when specimens are collected that show minor morphological differences (i.e. lighter or smaller) from the parent species, but are found outside of the expected species'

range (Chamberlin and Ivie 1942, 1947). Because subspecies are so rarely used in spider taxonomy, I feel it is an unnecessary designation and that it will not remedy the difficulty of correct identification of *groenlandica* species complex members.

Another limitation was an inability to create a comprehensive morphometric or morphology dataset for the use of a morphological phylogenetic analysis from the same specimens that could be later used for the molecular phylogenetic analysis. This is due in part because no morphologic work was originally planned, so specimens were collected for the purpose of molecular work, and the morphological analysis was only added after molecular work had been done.The collection of spiders directly into 100% EtOH for the preservation of DNA (Vink *et al.* 2005) caused some tissues to become brittle and shrink. Because of this, legs, spines, and trichobothria often broke, limiting the use of any features associated with them. Also, softer tissues associated with the abdomen contracted, and it is not known how internal tissues associated with the book lungs or reproductive organs might have been altered. Specimens were also kept cold (-80° C), which limits the ability to examine them. If at some point no further genetic use was needed from the specimens they could be rehydrated to 75% EtOH, at which time they would become flexible enough for a larger morphometric character set to be used, although it is not known how rehydration affects sensitive tissues like spines and trichobothria. No morphological phylogenetics have been done on any members of *Pardosa.*

Conclusion

All members of the newly arranged *groenlandica* species complex (*P. albomaculata, P. lowriei, P. bucklei* and *P. groenlandica*) can be identified

morphologically if *P. dromaea, P. tristis,* and *P. prosaica* are considered synonyms of *P. groenlandica.* This species complex shows large amounts of within-species and within-population genital variation, which has caused difficulty in the correct identification of members of the complex in the past. It is hoped that this work alleviates some prior identification problems within the species complex.

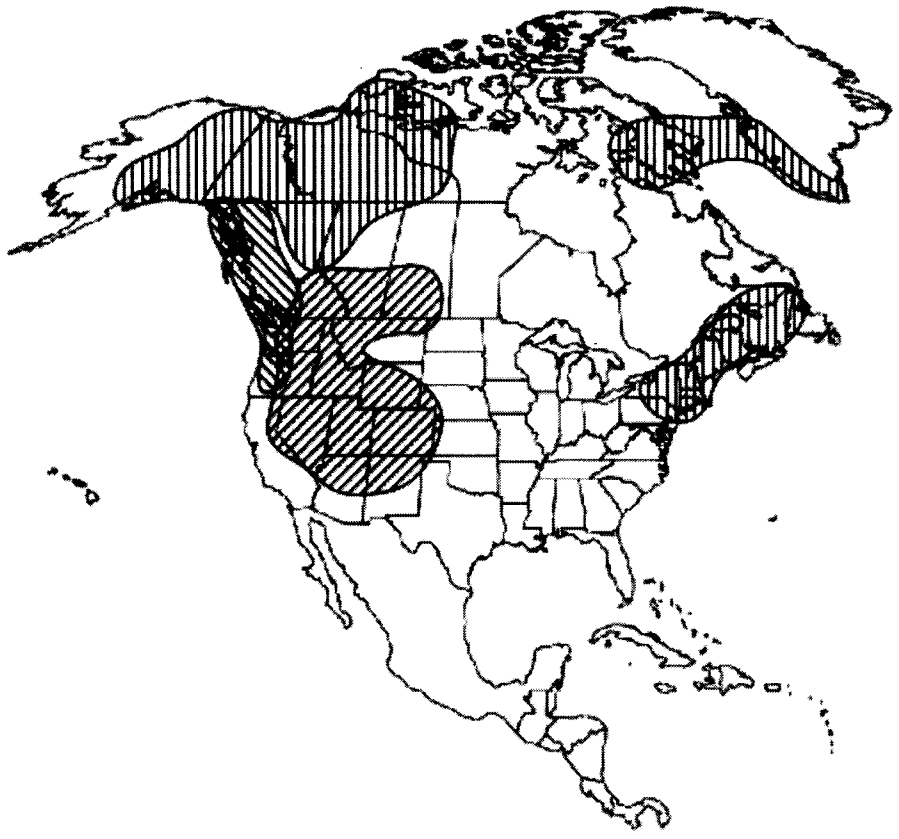


Figure 1.1 - Estimated distribution map for *Pardosa albomaculata* (vertical stripes), *P. lowriei* (stripes angle to the left), and *P. bucklei* (stripes angle to the right) based on Dondale and Redner (1990) and Dondale (1999).

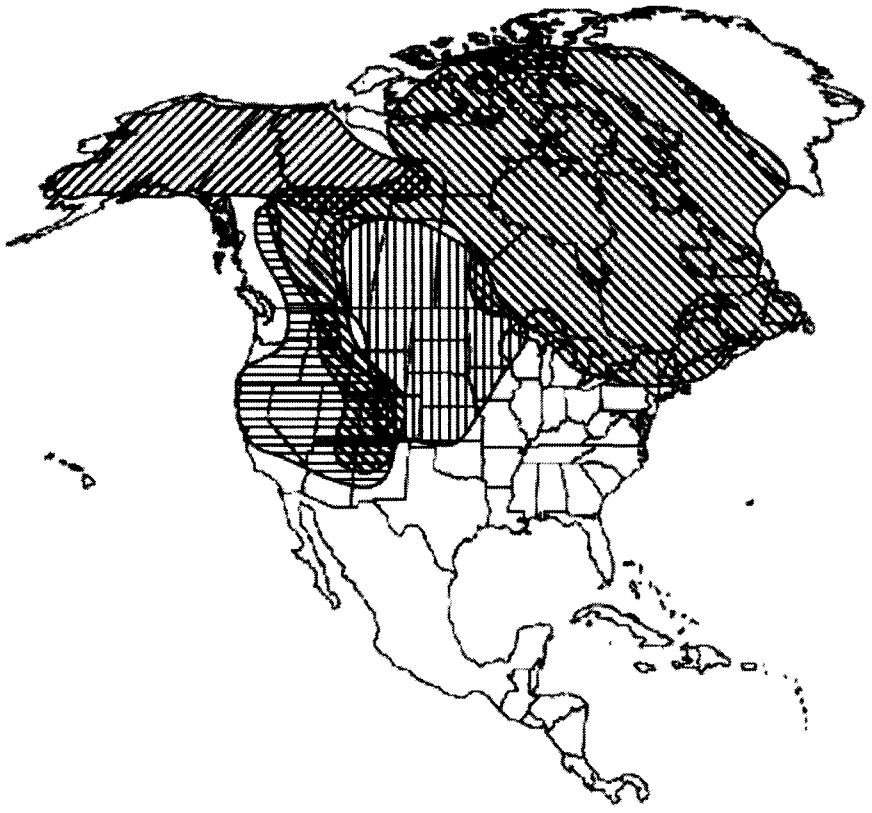
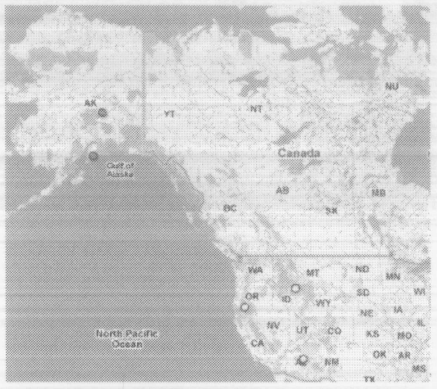
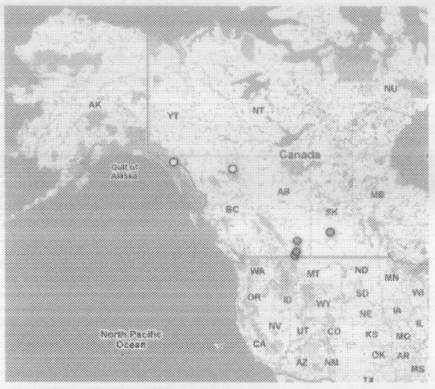


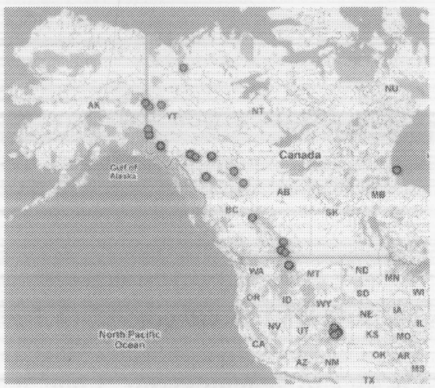
Figure 1.2 - Estimated distribution map for *Pardosa groenlandica* (stripes angle to the left), *P. dromaea* (vertical stripes), *P. tristis* (horizontal stripes), and *P. prosaica* (stripes angle to the right) based on Dondale and Redner (1990) and Dondale (1999).



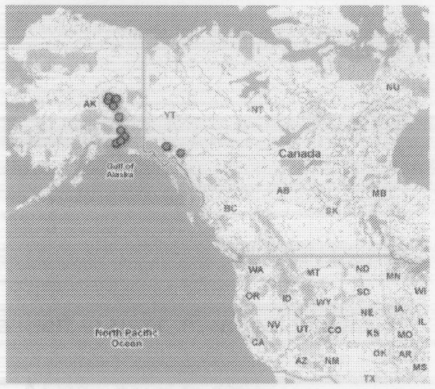
Pardosa albomaculata (Dark circles); *P. bucklei* (Light circles)



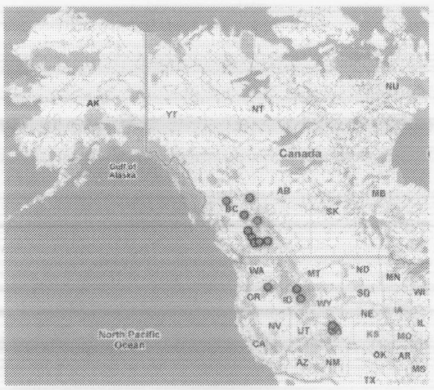
Pardosa dromaea (Dark circles); *P. lowriei* (Light Circles)



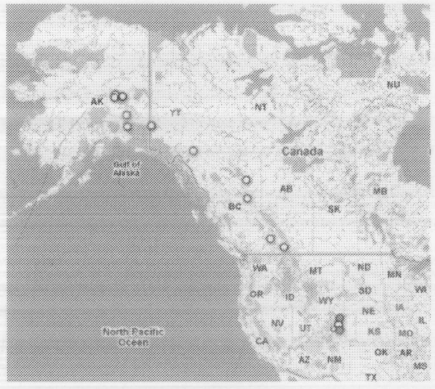
Pardosa groenlandica



Pardosa prosaica



Pardosa tristis



Pardosa xerampelina (Light circles); *P. ourayensis* (Dark circles)

Figure 1.3 - Collection localities of *Pardosa groenlandica* species complex specimens used in this study.

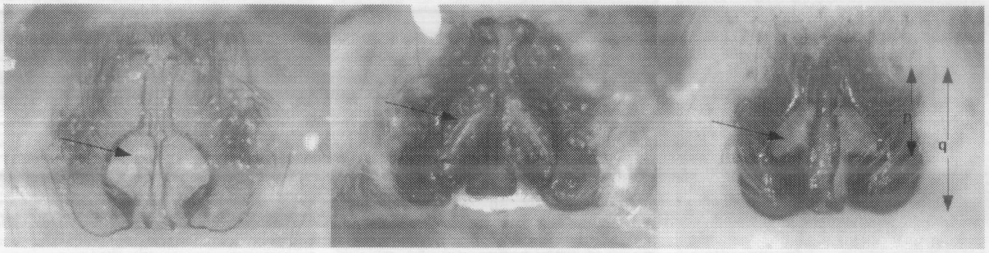


Figure 1.4 - Micrographs of *Pardosa* epigyna. Left to right, *P. albomaculata, P. lowriei,* and *P. groenlandica*. Images at 63 X. Arrows point to atria.

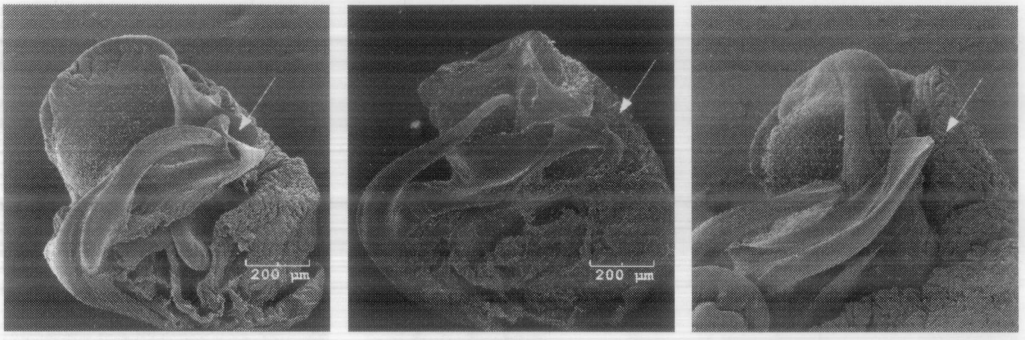


Figure 1.5 - Scanning electron micrographs of *Pardosa* palpal apical division. Left to right, *Pardosa albomaculata, P. lowriei,* and *P. groenlandica*. Images at 130 X. Arrows point to conductors.

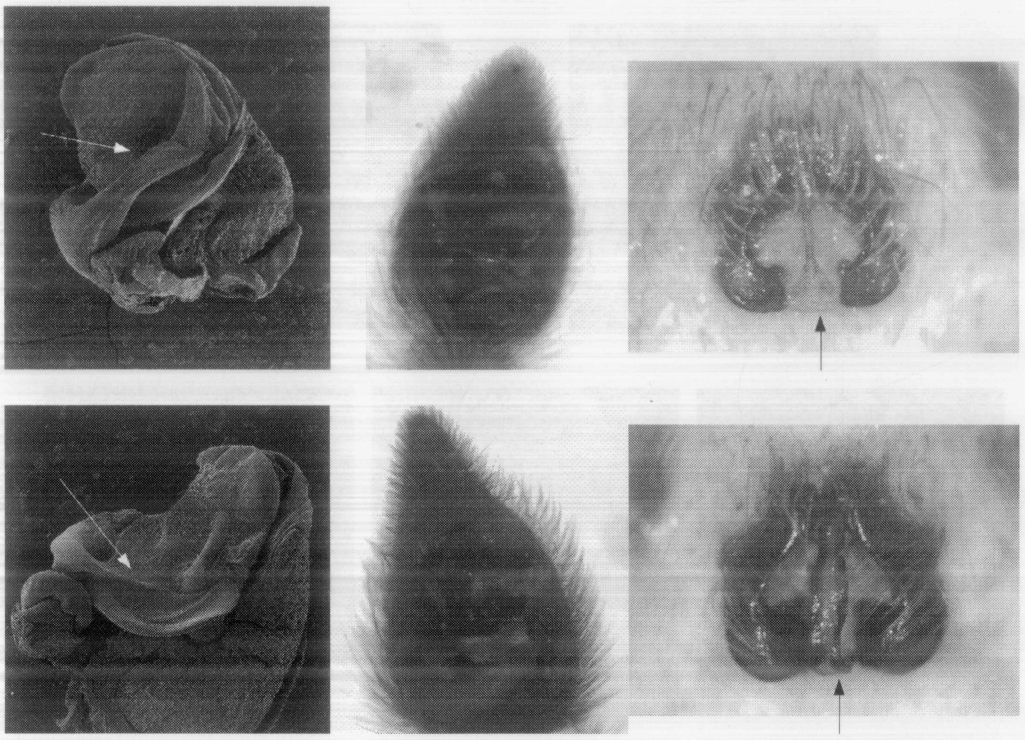


Figure 1.6 - Micrographs of *Pardosa* palps and epigyna. *Pardosa bucklei* top row, *P. groenlandica* bottom row. Left to right; Scanning electron micrograph of dissected palpal apical division (130 X), arrow points to embolus; micrograph of ventral view of palp (63 X *P. bucklei,* 50 X *P. groenlandica*); epigyna (80 X *P. bucklei,* 63 X *P. groenlandica*), arrow points to median septum.

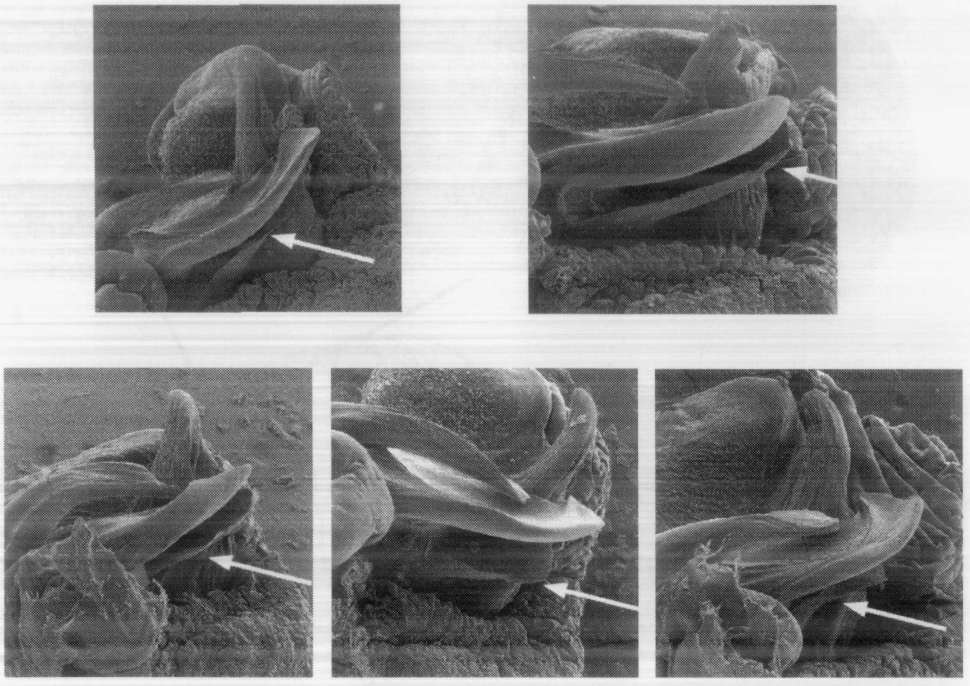


Figure 1.7 - Scanning electron micrographs of *Pardosa* palpal apical division. Clockwise from upper left; *P. groenlandica, P. tristis, P. dromaea, P. prosaica,* and *P. bucklei*. Images 130 X except for *P. bucklei* 190 X. Arrows point to retrolateral process of the terminal apophysis (RPTA).

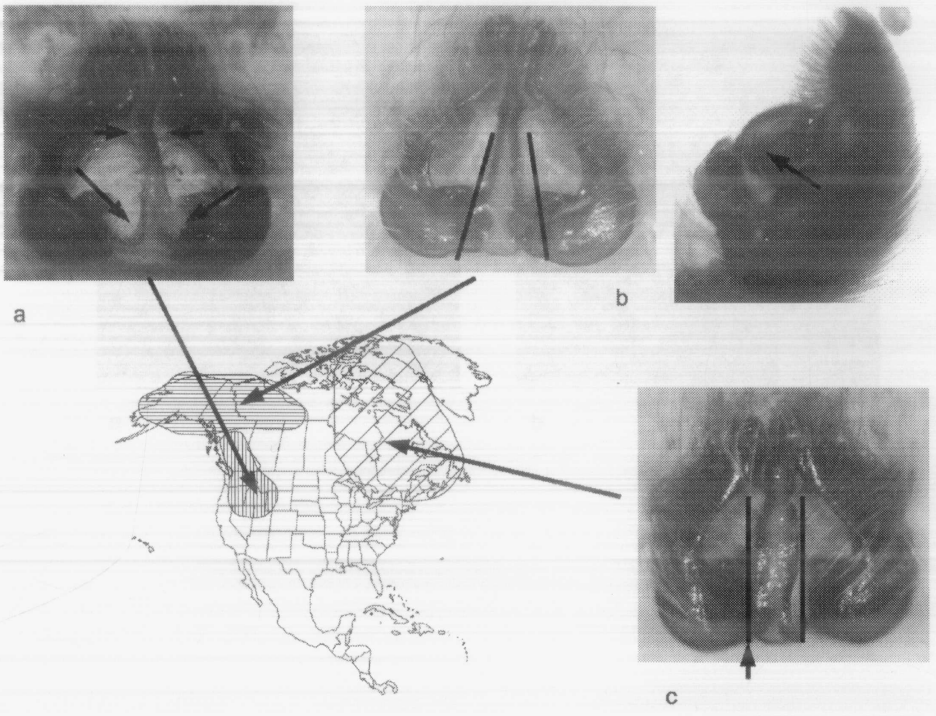


Figure 1.8 - *Sensu strico* morphology and geographic distribution of three *Pardosa groenlandica* species. a) *Pardosa tristis*, inverted T shaped median septum (MS), arrows point to narrow anterior and posterior regions. b) *P. prosaica*, left image - urn shaped MS, lines emphasize widening of MS posteriorly; right image - palp, retrolateral view, arrow points to constriction on interior edge of the embolus. c) *P. groenlandica,* lines emphasize parallel shape of MS lateral edges.

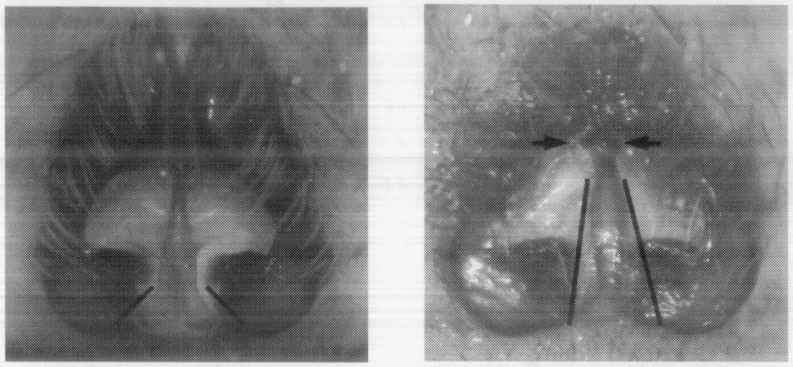


Figure 1.9 - Additional median septum (MS) shapes of *Pardosa groenlandica* species complex specimens. a) "A" shaped MS, lines emphasize abrupt posterior widening of MS. b) Example of MS showing a conglomeration of characters, arrows point to wider anterior region similar to a bottle shaped MS, lines emphasize posterior swelling of MS similar to an urn shaped MS.

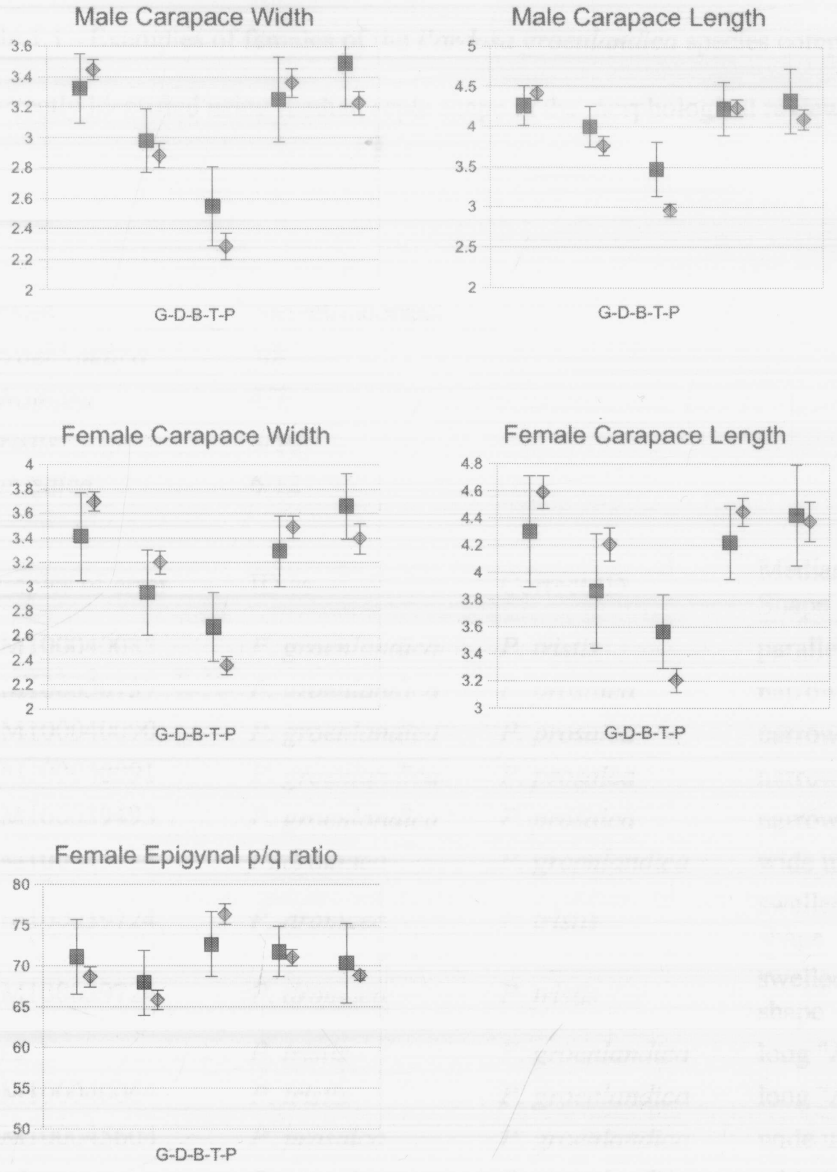


Figure 1.10 - Graphical comparison of morphometric characters used in Dondale (1999) with those collected in this study. Squares are Dondale's data, diamonds results from this study. Error bars are standard errors. Y-axis is measured mm, X-axis refers to order of species and corresponding column. G-*Pardosa groenlandica*; D-*P. dromaea*; B-*P. bucklei*; T-*P. tristis*; P-*P. prosaica*.

Table 1.1 - Examples of females of the *Pardosa groenlandica* species complex incorrectly identified using median septa shape in the morphological review.

Females	correct/incorrect
P. groenlandica	3/8
P. dromaea	4/7
P. tristis	8/10
P. prosaica	6/12

Examples of error	ID as	Correct ID	Median Septa Shape
UAM100040085	*P. groenlandica*	*P. tristis*	parallel urn shape
UAM100050737	*P. groenlandica*	*P. prosaica*	narrow urn shape
UAM100040090	*P. groenlandica*	*P. prosaica*	narrow urn shape
UAM100040091	*P. groenlandica*	*P. prosaica*	narrow urn shape
UAM100039493	*P. groenlandica*	*P. prosaica*	narrow urn shape
UAM100045725	*P. dromaea*	*P. groenlandica*	wide urn shape
UAM100039724	*P. dromaea*	*P. tristis*	swelled "A" shape
UAM100039724	*P. dromaea*	*P. tristis*	swelled "A" shape
Chu 5	*P. tristis*	*P. groenlandica*	long "A" shape
UAM100040064	*P. tristis*	*P. groenlandica*	long "A" shape
UAM100045604	*P. prosaica*	*P. groenlandica*	wide urn shape
Chu 4	*P. prosaica*	*P. groenlandica*	wide urn shape
UAM100045726	*P. prosaica*	*P. groenlandica*	wide urn shape
UAM100045706	*P. prosaica*	*P. groenlandica*	wide urn shape
Chu 10	*P. prosaica*	*P. groenlandica*	wide urn shape
UAM100045758	*P. prosaica*	*P. tristis*	"A" shape

Table 1.2 - Measurements (in mm) of the *Pardosa groenlandica* species complex. Means significantly different from other species are identified by the first letter of the species name. Asterisk signifies measurements significantly different from Dondale 1999.

	Carapace Width				Carapace Length					
Species	Male	se	Female	se	Male	se	Female	se	Epigynal ratio	se
P. groenlandica	3.44	0.07	3.69	0.08	4.42	0.08	4.59	0.12	68.64	1.27
P. dromaea	2.88	0.08 bgtp	3.2	0.1 bgtp	3.76	0.12 bgtp	4.21	0.12	65.82	1.19 gdtp
P. bucklei	2.29	0.09 gdtp	2.35	0.08 gdtp	2.96	0.07 gdtp*	3.2	0.09 gdtp	76.29	1.25 gdtp
P. tristis	3.36	0.09	3.49	0.09	4.23	0.11	4.45	0.1	71	1.03
P. prosaica	3.23	0.07	3.39	0.12	4.09	0.12	4.37	0.15	68.8	0.61

Chapter 2

Phylogenetic systematics of the *Pardosa groenlandica* species complex

Abstract

The systematic relationships of taxa in the *Pardosa groenlandica* species complex was analyzed using partitioned Bayesian analyses of the mitochondrial DNA genes COI and ND1 and the nuclear DNA genes ITS1, 5.8S, ITS2, and Actin5C. Additionally, spiders were also DNA barcoded. These results were examined for evidence supporting either the seven- or four-species assemblages for the *P. groenlandica* species complex (Chapter 1). The phylogenetic analyses found no conclusive support for either the seven- or four-species assemblage. Rather, gene-based phylogenies did not match species designated by morphology. The mitochondrial DNA were analyzed for phylogeographic evidence, because these spiders can be found in areas of North America that were glaciated during the last glacial maximum. The phylogeographic evidence was inconclusive;specimens from similar geographic areas do form distinct clades, however, other specimens of the same species collected from a single collection event did not cluster with one another. Currently defined species in the *P. groenlandica* group show various amounts of genetic support, with a general lack of agreement between genetics and morphology for species boundaries.

Introduction

Molecular studies have been conducted on difficult spider species complexes, often with a great deal of success in delineating species and finding cryptic species (Paquin and Hedin 2004, Hendrixson and Bond 2005, Bond and Stockman 2008, Huber *et al.* 2010). Recent work with DNA barcoding of spiders has also demonstrated the value of molecular information in species delineation and identification (Barrett and Hebert 2005, Robinson *et al.* 2009). However, molecular studies using a single gene are susceptible to misinterpretation as only a single evolutionary path is examined (Maddison 1997, Paquin and Hedin 2004). Single-gene analyses often use a mitochondrial gene as these have shown tremendous versatility in identifying lineages and phylogeographic trends (Avise 2004, Ballard and Rand 2005). There are many more copies of the mitochondrial genome present in an organism, allowing for more successful sequencing compared to nuclear genes. But the use of only mitochondrial genes is limiting. It is now generally agreed that the evolutionary forces affecting mitochondria are similar (e.g., mtDNA is a single locus), such that adding additional mitochondrial genes only provides additional information of the mitochondria's evolutionary lineage. Mitochondrial genes are also subject to unseen error in the form of nuclear copies of those genes, often referred to as numts (den Tex *et al.* 2010).

Adding nuclear genes to an analysis provides loci independent of mtDNA, but is often limited as nuclear genes generally are more conserved than those of the mitochondria, and thus generally have less phylogenetic information per sequenced

base pair. The ribosomal DNA (rDNA) region around the 18s, 5.8S, and 28s subunits, particularly the internal transcribed spacer (ITS) sections between the subunits have shown utility for species-level analyses (Agnarsson 2010). However, the amount of change in these ITS regions is not predictable as they are a series of tandem repeats susceptible to indel events which may show large changes between and among species (Bower *et al.* 2008) or few changes among species (Vink *et al.* 2008). In these conserved cases the secondary structure may be informative for delineating species (Coleman 2009). Additionally, rDNA sequences are more likely to be contaminants than mtDNA because the ribosomal subunits are highly conserved across eukaryotic animals (Vink *et al.* 2008, Muster *et al.* 2009, Coleman 2009) allowing primers to amplify even disparate phyla.

Besides assisting in species delineation, molecular data can be very effective in revealing phylogeographic histories. Phylogeographic evidence exists in both plants and animals for late Pleistocene refugia in central Alaska to Greenland, i.e. Beringia (Federov and Stenseth 2002), the Pacific Northwest (Demboski *et al.* 1999), and for refugia further south in central North America (Aubry *et al.* 2009). The distribution of the species in the *Pardosa groenlandica* species complex currently includes areas that would have been refugia during the end of the Pleistocene period. However, because precise habitat requirements are not known for any of the species, it is not clear how range expansions or contractions would have been affected by the changing climate.

Chapter 1 demonstrated that species of the *Pardosa groenlandica* species complex are morphologically very similar, but that they also show a great deal of

morphological variation among species and populations. Because of this, species delineation based on morphology and distribution is difficult, resulting in two likely species assemblage hypotheses for the species complex. These are the seven-species hypothesis proposed by Dondale (1999) and the four-species hypothesis proposed in Chapter 1. Here I use mitochondrial and nuclear loci to examine the likelihood of either of these proposed species assemblage hypotheses. Additionally, mtDNA data are used to examine the phylogeographic history of the species complex and the possibility of speciation due to vicariance during the late Pleistocene.

Methods

Gene Selection

Molecular data were generated for the mitochondrial genes COI and NDI and the nuclear genes ITS1, the ribosome 5.8S (hereafter referred to as just 5.8S), ITS2, and Actin5C. The mitochondrial genes COI and NDI were selected because of their demonstrated value in species-level separation in spiders (Muster and Berendonk 2006, Barrett and Hebert 2005, Robinson *et al.* 2009). The nuclear ITS region genes were selected because they have been found to contain inter- and intra-species information in other phylogenetic analyses of spider taxa (Chang *et al.* 2007, Bond and Stockman 2008, Vink *et al.* 2008, Muster *et al.* 2009). The Actin5C gene has not yet been examined for intraspecies relationships in spiders (Vink *et al.* 2008).

DNA Extraction and PCR

Extractions of 182 fresh specimens (Appendix 1) were made using the right

third leg of the spider and done in the PCR-free DNA lab at the University of Alaska Museum (see Chapter 1 for details on specimen collection and preservation). Two outgroup species were selected: *P. ourayensis* which is in the *modica* group of *Pardosa* with the *groenlandica* species complex, and *P. xerampelina* which is a non-*modica* group species. DNA was extracted using a Qiagen DNeasy Tissue Kit (www.qiagen.com) following the manufacturer's instructions except for: 100% ethanol at -80°C was used rather than at room temperature, and two 100 µl elutions using 65°C AE buffer were performed rather two 200 µl elutions using room-temperature AE buffer. These adjustments were found to increase DNA yields. Adding legs did not greatly increase the yield. Extractions were quantified using a Nanodrop ND-1000 spectrophotometer (Thermo scientific, Wilmington, DE) and generally ranged around 10 ng/µl.

Polymerase chain reaction (PCR) was conducted at the DNA CORE lab, University of Alaska, Fairbanks. PCR programs, primers, and mixtures varied (Table 2.1). PCR mixtures were 25 µl in volume for the Actin5C, NDI, ITS1, ITS2, 5.8S genes and 30 µl for the COI gene, of which 12.5 µl was GoTaq master mix (www.promega.com) as recommended by the manufacturer. PCR was conducted using a PTC-255 thermocycler (MJ Research Peltier). All PCR temperature profiles had an initial separation stage of 94°C for 4 min; 50 cycles of 30 s at 94°C, 30 s or 45 s at the designated annealing temperature (Table 2.1), 1 min at 72°C; and a final extension stage of 72°C for 10 min. Verification of PCR products was made using a 20 ml, 2% argrose gel infused with 2 µl of ethidium bromide (EtBr). Bands were visually evaluated for DNA concentration and were in the area of 10-20 ng/µl. PCR

cleanup and sequencing were done at the High-Throughput Genomics Unit at the University of Washington (www.htseq.org).

Sequence Assembly and Alignment

Contiguous sequences were assembled from bidirectional primer reads. A consensus sequence was assembled from the forward and reverse complement sequences and aligned by eye. Initially, Codon Code (www.codoncode.com) was used, but because this software is not open source, future replication could be hindered. To alleviate this, a similar method was conducted using 4peaks v 1.7.2 (http://mekentosj.com) to survey the chromatograms, and MESQUITE v 2.72 (Maddison and Maddison 2009, www.mesquiteproject.org) was used to create contiguous sequences and check for ambiguities. Sequences were aligned for each gene using Clustal X v 2.0.12 (Thompson *et al.* 1997) and then visually checked for odd gaps and alignment errors against chromatograms. The mitochondrial DNA alignment was verified by translation to amino acid sequences and comparison to amino acid sequences AAX40574.1 and ABF59654.1 obtained from GenBank (www.ncbi.nlm.nih.gov/Genbank/index.html). ITS data were anchored at the conserved 5.8S ribosome region and aligned moving away from this region.

Phylogenetic Analyses

Individual genes were evaluated using Modeltest 3.7 (Posada and Crandall 1998) implemented in PAUP* v 4.0b10 (Swofford 2002) to determine which available phylogenetic model best fit each. For the COI data an independent TrN+I+G

model was selected for each codon position. For the NDI data an independent GTR+I+G model was selected for each codon position. For the ITS1 data HKY+I+G model was selected; K80 was selected for the 5.8S data, and F81 was selected for the ITS2 data. Because MrBayes cannot implement the TrN model, the GTR model was used instead. Bayesian phylogenetic analyses were conducted using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). I used each of the four genes as an individual dataset in addition to running a combined dataset in a partitioned analysis to infer a species tree from the respective gene trees (Nylander *et al.* 2004). To accommodate for branch length estimation errors made by MrBayes' algorithm when dealing with partitioned datasets, I changed the branch length prior from the default value of brlenspr=unconstrained:exponential(10) to brlenspr=unconstrained:exponential(100) as recommended by Marshall (2010) and Brown *et al.* (2010). This modification brought the branch length estimates close to those of an unpartitioned analysis using the GTR+I+G model with GARLI v 0.96 (Zwickl 2006).

Several different partitioned analyses were conducted in an attempt to find the best overall partitioning scheme (Table 2.2). How one selects the best partitioning scheme for their data remains an area of current research (Li *et al.* 2010, Klopfstein *et al.* 2010, Ward *et al.* 2010). For this study, Bayes' factors were used to determine which partitioning scheme was the most informative (Kass and Raferty 1995, Nylander *et al.* 2004, Ward *et al.* 2010).

Bayesian MCMC chains were initially run for 2,000,000 generations, keeping every 1000th tree. The default setting of two simultaneous runs with four chains each run was used. If stationarity was not achieved during this period, the number of

generations was increased to 4,000,000, and if stationarity was still not reached a final analysis of 10,000,000 generations was run. Stationarity was determined using three factors: 1. Split frequencies were at or below 0.01 (Ronquist and Huesenbeck 2003). 2. Visual inspection of trace files confirmed a plateau was reached for at least 50% of the generations (Ronquist and Huesenbeck 2003). 3. ESS (Effective Sample Size) values for all model parameters were over 100. The effective sample size is the number of effectively independent samples taken from the MCMC chains and is usually much smaller than the number of samples due to autocorrelation (non-independence) among samples (Drummond *et al.* 2006). Trace files were inspected using Tracer v 1.4 (http://beast.bio.ed.ac.uk/Main_Page).

The initial 50% of the trees of each run were removed as a burn-in period to ensure that only data post-stationarity were included in tree reconstruction. A 0.90 posterior probability consensus tree was assembled using Sumtrees 2.0.2 (Sukumaran and Holder 2010). All phylogenetic analyses were run using the Life Science Informatics Cluster at the University of Alaska, Fairbanks (https://biotech.inbre.alaska.edu). An additional analysis of 100 bootstrap replicates using a GTR+I+G model with 2,000,000 generations per replicate was conduced with Garli v 0.96 to provide a maximum likelihood comparison. Genetic diversity for each gene was calculated from uncorrected *p*-value distances calculated using Arlequin v 3.1 (Excoffier *et al.* 2005). Uncorrected *p*-value distances were calculated for the combined dataset using PAUP* v4.0b10 (Sinauer Associates).

DNA Barcode Identification

To attempt species identification using DNA barcoding, the 5-prime half of the COI data that matches the recommended DNA barcode section was analyzed (Barrett and Hebert 2005, Robinson *et al.* 2009). Sequences were obtained from the BOLD database and used to locate the correct section of COI to be used for comparison. A neighbor joining tree was made with K2P distances using PAUP*.

Results

Phylogenetic Analyses

Sequences were generated for the COI, ITS1, 5.8S, and ITS2 genes for 144 specimens, for the NDI gene for 13 specimens, and for the Actin5C gene for six specimens. Details on the nucleotide composition, sequencing success, and variable site numbers for each gene can be found in Appendix 2. Because of the low number of sequences obtained for the Actin5C gene, no phylogenetic interpretation was made using those data. A combined dataset for use in the partitioned analysis was made of the 144 specimens that were successfully sequenced for the COI, ITS1, 5.8S, and ITS2 (rDNA) genes. For the purpose of comparing the two different species assemblage hypotheses, all trees (Figures 2.1-2.8) show specimens as identified using the *sensu stricto* descriptions in Dondale (1999), which represents the seven-species hypothesis; specimens that would be synonymized under *P. groenlandica* in the four-species hypothesis are highlighted as grey text. The general geographic position of each specimen and its applicable population designation (Table 2.3) is recorded using

United States and Canadian state and province postal codes at the end of each specimen.

Analysis of COI showed that the branch separating the outgroup *P. xerampelina* from the rest of the samples was well supported (Figure 2.1). However, support within the ingroup was mixed. There was strong support for the *P. ourayensis* and *P. albomaculata* clades, but their relationships within the *modica* group were not clear because of a basal group consisting of 13 specimens, representing five species, covering the entire sampled geographic range (Figure 8, grey highlight, Appendix B). This basal group is also supported in the NDI data (Figure 2.2, grey highlight). Multiple extractions and PCR amplifications were conducted on some specimens to verify the basal group (Appendix 1 & 2). The COI data also supported a *P. lowriei* clade; however, the species was not monophyletic, with one member falling out in the basal group. There was a general grouping congruent with geography, which was expected from mtDNA sequence data (Avise 2004). This geographic grouping was most obvious in clades of *P. prosaica* and *P. groenlandica* from Alaska and Yukon and *P. tristis* from British Columbia. However, there were several results that contradicted this pattern. Specimens of *P. groenlandica* from Manitoba (MB) were all collected from a single population 1300 km from the next closest sample. These samples were polyphyletic. Also, all samples of *P. bucklei* were from a single population and also appeared polyphyletic in the results.

Phylogenetic analyses of the ITS2 and 5.8S RNA genes resulted in polytomies, which was to be expected based on the few informative sites (1 parsimony-informative site) available for inference. ITS1 results (Figure 2.3)

supported the exclusion of both outgroup species from the species complex. *Pardosa xerampelina* was monophyletic in the ITS1 results, ignoring specimen UAM100045687. All *P. xerampelina* specimens were found to have a TA base insertion 111 bases in the 5' direction of the 5.8S subunit. The ITS1 results also showed strong support for a terminal *P. ourayensis* group. *Pardosa ourayensis* specimens also showed an extra A at base 400 and an extra G at base 396 in the 5' direction from the 5.8S subunit, which were not found in any other specimens. Each population of *P. lowriei* was monophyletic, but the species is polyphyletic. The rDNA data did not cluster specimens of *P. albomaculata,* which was monoplyetic in the COI data. Because no species was found to be monophyletic, the rDNA data failed to clearly support either the four- or seven-species hypothesis.

Determining run completion and an optimal partitioning scheme was difficult for the combined analysis. No partition scheme even after 10 million generations achieved split frequencies below 0.01 as recommended in the MrBayes manual (Table 2.4). Additionally, not all parameter values achieved ESS values over 100, although log-likelihoods of all runs except for p6 had ESS values over 100. Because of this, Bayes factors were calculated twice, using the combined runs' harmonic means, and also the best harmonic mean of the two runs. Bayes factor results implied that there was little improvement beyond the p4i scheme (Table 2.4). This scheme allowed for codon positions in the COI gene but used the same model (HKY+I+G) across all of the nuclear data. A slight improvement was seen moving to a five- (p5) or six- (p6i) parameter model. The six-parameter model (p6) without the branch-length correction never stabilized (ESS of log-likelihood value not over 100), even though the

likelihood value was similar to that of the four-parameter value after 10,000,000 generations.

Well supported, 0.90 posterior probability, topologies of the four (p4i)-, five (p5)-, and six (p6i)-partition schemes were not identical (Figures 2.4-2.6). Both runs of the four- and six-partition schemes had similar topologies; however, the two runs of the five-partition scheme were either similar to the four-partition topology or the six-partition topology (not shown), resulting in an intermediate consensus tree. It would appear that the biggest discrepancy was in how the partition scheme treated the ITS1 data and the COI basal group. The ITS data (Figure 2.3) strongly supported the placement of *P. ourayensis* as sister to *P. groenlandica* WE3, whereas the COI data supported the basal group and also the separation of the *P. albomaculata* clade (Figure 8).

The likelihood bootstrap analysis showed less resolution than any of the partitioned Bayesian analyses (Figure 2.7). This analysis supported both the *P. ourayensis* and *P. lowriei* clades, minus specimen UAM100039659, but not the basal clade seen in the COI data or the Pacific Northwest clade seen in the four- or six-partition schemes (Figures 2.4 and 2.6).

Genetic Variation

Nucleotide variation of the COI gene, using uncorrected *p*-distances, ranged in the ingroup from 0.31% in *P. albomaculata* to 2.25% in *P. bucklei*, and was within previously published ranges found in other spider species (0%-3.6%, Barrett and Hebert 2005, 1.3%-5.7%, Crews *et al.* 2009). Variations in the ITS1 and ITS2 genes

(0.02%-1.1%) were also similar to previously published values (0.6% Chang *et al.* 2007; 2% Muster *et al.* 2009).

Total uncorrected *p*-distances for the combined data showed that members of the *groenlandica* species complex are more closely related than either of the two outgroup species, *P. xerampelina* and *P. ourayensis* (Table 2.5). Within-species variation was not lower than between-species variation for *P. bucklei*, which was also apparent in the species being polyphyletic (Table 2.5, Figures 2.1-2.7).

Barcode identifications

My COI data, in relation to those of the BOLD database, had 428-590 bases of overlap of the possible 660 bases. The neighbor-joining tree failed to show clear species-level monophyly for any of the seven members of the *groenlandica* species complex (Figure 2.8). Both *P. albomaculata* and four of the specimens of *P. lowriei* showed long branches that suggest further inquiry may be warranted, but these results do not clearly identify those species as both are either paraphyletic or polyphyletic. Identification using these results also failed to identify the outgroup species *P. ourayensis*, but they did identify *P. xerampelina,* although the long branches separating two of the four specimens calls for further inquiry into the possibility of a cryptic species.

Using the BOLD identification engine for species identification resulted in the correct identification of *P. xerampelina,* but misidentified *P. ourayensis* as *P. modica* (Blackwall 1846). All other specimens were identified as *P. groenlandica.* These results are to be expected as currently there are no publicly available sequences for

any of the species used in this study besides *P. groenlandica* and *P. xerampelina*.

Phylogeography

Phylogeographic interpretation of the COI results showed strong support for a large Alaska/Yukon cluster and a British Columbian cluster, from which *P. lowriei* appears to have originated (Figure 8). There is also support for several other clusters at the population level with some mixing of various populations. In this tree (Figure 2.1), branch lengths were of similar length in many of the geographically distant, strongly supported clades. The strongly supported clades do not appear to originate from an ancestral population corresponding to any of the hypothesized refugia. The rDNA results did not support any possible geographic clusters or glacial refugia.

Discussion

These results did not show strong monophyletic support for any of the species included in the *Pardosa groenlandica* species complex. This is somewhat peculiar as a majority of molecular studies do find concordance with some level of molecular monophyly and existing species (Pons *et al.* 2006, Yang and Rannala 2010). Additionally, molecular studies are finding additional lineages not identified using traditional taxonomic methods (Paquin and Hedin 2004, Lopez *et al.* 2007). Because of the interest in the identification of species using a DNA barcoding approach there are now numerous examples of molecular phylogenies of the COI gene. Most of these do work well in identifying species using a single locus (e.g. Cywinska *et al.* 2006, Robinson *et al.* 2009). However, there are a growing number of studies that find the

molecular phylogeny discordant with the current taxonomy (e.g. Cognato 2006, Schmidt and Sperling 2008). As the number of molecular studies continues to grow, so do the number of studies finding discordance, in which species are found to be polyphyletic or paraphyletic. A study by Funk and Omland (2003) found that 23% of species showed some level of polyphyly or paraphyly based on molecular data. It should be noted that a gene tree may not match the species tree, because different genes evolve individually and may show different phylogenetic histories (Maddison 1997). These results can be due to many factors, such as mtDNA fixation due to *Wolbachia* (Whitworth *et al.* 2007), hybridization (Lopez *et al.* 2007, Schmidt and Sperling 2008), incomplete lineage sorting, selective sweeps (or mtDNA capture), gene flow, and incorrect taxonomy (Hendrixson and Bond 2005). False monophyly can also be inferred due to a species' vagility, geography of the samples, or sample size (Rosenberg 2007), in addition to methodological errors in the lab or analyses.

The close likelihood scores of the four- (p4i), five- (p5) and six (p6i)-partition models make interpretation of the best phylogenetic partition scheme difficult. Although the Bayes factor comparison found the six-partition model marginally better than the five-partition model (p5), the best likelihood score was in run 1 of the five-partition model. However, the increase in likelihood score with the more complex models, five or six over four, may not warrant the additional parameters. The results of the four-, five-, and six-partition models did differ slightly in topologies (Figures 2.5-2.6). In all three, the outgroup species were supported, and four of the five *P. lowriei* specimens did group together. The basal group found in the COI data was not recovered in the five-partition model but was in the four- and six-partition models,

although not it was not basal in the four-partition model. The slight differences in the topologies of the best three-partition schemes also did not clarify the species question, as none of the morphologically supported species complex members were strongly supported phylogenetically.

If it is assumed that the speciation episode(s) of the species complex occurred during one or over several of the recent glacial cycles, it follows that an ancestral population(s) would have existed in one or several of the available refugia. Although the COI data do support some geographic clusters, none of these clusters appear as a source population that may have survived the Pleistocene glaciation for the species complex. Both the Alaska/Yukon and British Columbian clusters show large geographic coverage with limited genetic variability, implying that both are recent range expansions, but it is unclear where these populations originated. Also supported is the speciation of *P. lowriei* from the British Columbian cluster. There are conflicting opinions as to the extent of the Pacific Northwest's refugia. One hypothesis is for a coastal as well as a continental refugium (Byun *et al.* 1997), whereas other evidence seems to contradict this, supporting a single refugium (Demboski *et al.* 1999). If there was a coastal refugium, this may have acted as the vicariance episode allowing for the speciation of *P. lowriei.* The more southern and eastern populations of species complex members showed higher mtDNA variability, but are not restricted to a particular area. Without additional specimens from the full extent of the species complex's range it is difficult to make generalizations about gene flow. It is also difficult to interpret the origin of the basal cluster as this group shows no geographic structure.

A Pleistocene speciation episode would put the time frame of speciation somewhere between 10,000-50,000 years ago (Brunsfield *et al.* 2001). If the members of the *groenlandica* species complex are this young, it would not be expected for them to show large amounts of genetic divergence, as seen in the low amount of phylogenetic information in the rDNA. Additionally, traditional phylogenetic analysis had been found to fail to correctly identify species of recent divergence (Knowles and Yang 2008).

It is unfortunate that no specimens could be obtained for molecular analysis from Greenland or the East Coast of North America which would correspond to the *sensu stricto Pardosa groenlandica.* These specimens might provide evidence of geographic gene flow and historic populations not found in this phylogenetic analysis, and would aid in the ability to examine the phylogeographic patterns of the species complex. Additionally, the fact that the type locations of *P. tristis* and *P. dromaea* no longer retain populations of either species is troublesome as there remains doubt that the correct species have been molecularly sampled.

Despite being found effective for species or population delineation in other spider studies (Robinson et al. 2009, Agnarsson 2010), including other species of *Pardosa* (Chang et al. 2007, Muster and Berendonk 2006, Muster et al. 2009), there is the possibility that the genes used were simply uninformative for the *groenlandica* species complex. However, the number of parsimony informative sites of each gene used in my analyses was comparable to other studies in which species delineation was inferred (Crews et al. 2009, Bond and Stockman 2008). This suggests the explanation doesn't lie in the amount of genetic change but rather in the evolutionary processes

that produced the changes. Another problem may be the limited ability to model change in the rDNA region. Although the 5.8S ribosomal region is highly conserved, changes in the ITS1 and ITS2 regions are largely due to indel events (Coleman 2009, Bower et al. 2008), of which we have no adequate phylogenetic model (Rosenberg 2009).

Prior to this study no phylogenetic studies addressed species limits in *Pardosa.* There are several previous molecular studies of the genus (Muster and Berendonk 2006, Chang *et al.* 2007, Muster *et al.* 2009), but they involved a single species hypothesis and operated at the population level. The study of *Pardosa sierra* Banks 1898 (Correa-Ramirez *et al.* 2010) used morphology with genetic-distance comparisons in examining species delineation. However, their analyses inadequately tested the monophyly of each species due to limited sample size (only two specimens per species), and limited genetic information (only the 660 bases of the barcode region of the mtDNA COI gene). Previous DNA barcode data of *P. groenlandica* from Eastern North American samples was found to have a high amount of within-species variation (3.8%) and was thought to maybe represent a species complex. However, the limited genetic information of the DNA barcode region was not capable of resolving the variation observed (Robinson *et al.* 2009). Other studies within the Lycosidae have found monophyletic species lineages using several loci (Vink and Patterson 2003, Hosseini *et al.* 2007).

It is clear that there is structure in this dataset, just not structure indicative of several distinct monophyletic or even paraphyletic species. However, the assumption that all these samples represent one species is contradicted by the morphological

evidence that currently defines the species (Chapter 1). The structure of the phylogeny suggests the clades are of recent origin as all branch lengths are relatively short. Also apparent is the geographic population structure in the mtDNA (Figure 2.1), which implies that although these spiders possess the ability to disperse long distances when young via ballooning, many do not. It is impossible to conclusively determine which of the species assemblage hypotheses presented previously is most likely from these data. These data support neither the seven species of Dondale, nor the four species of Chapter 1. They also do not present a strongly supported alternative species assemblage hypothesis.

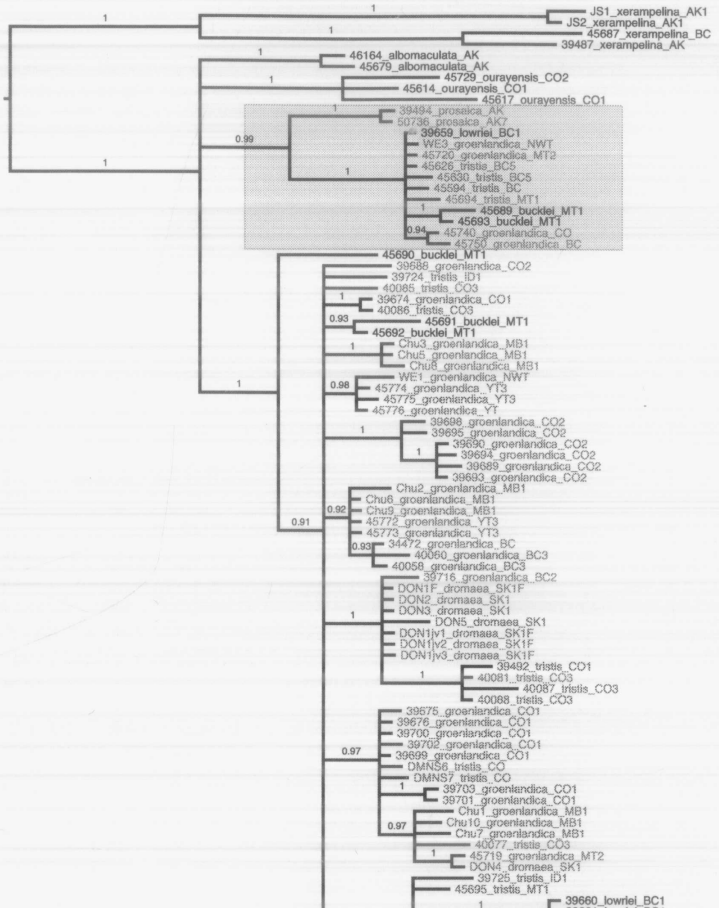
Although this study did include a large number of samples (n=144) over a wide geographic range there are certainly areas in which samples were not collected which may provide information. The most glaring omission is any *P. groenlandica* from Greenland, which might have provided evidence of whether *P. groenlandica* extends across North America as currently assumed or is limited to Greenland. Additionally, the low sample size and limited location sampling of *P. albomaculata, P. lowriei,* and *P. bucklei* also limit my ability to make any specific interpretations, because adding samples of other species complex members did produce separate geographic lineages (Figure 2.1). Because of this I feel that these results are too preliminary for any taxonomic interpretation.

Conclusion

These data and phylogenetic methods did not find any of the species of the *Pardosa groenlandica* species complex to be monophyletic. These analyses also

failed to find conclusive support for either the seven-species hypothesis of Dondale (1999) or the four-species hypothesis of Chapter 1. Perhaps adding additional loci, nucleotides, and specimens from a broader geographic range will help to alleviate these difficulties. Because these data are inconclusive about species boundaries, it remains up to the researcher to make the decision as to specimen identification, either retaining the previous taxonomy of Dondale (1999) or accepting the revised four-species taxonomy of Chapter 1. Also uncertain is the geographic history of the species complex. These data do find evidence for *P. lowriei* diverging from a British Columbian lineage of *P. tristis*, but they do not provide any clear evidence for speciation events for any of the other members of the species complex.

Figure 2.1 - Bayesian 90% majority rule consensus phylogram for species of the *Pardosa groenlandica* species complex and outgroup based on 1120 bp of the mitochondrial COI gene using a three partitioned GTR+I+G model for each codon position. Values above branches are posterior probabilities. The grey box highlights basal group, further explained in text. Grey specimens represent those which would be included in *P. groenlandica* based on morphological results of Chapter 1.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Chu6_groenlandica_MB1
Chu9_groenlandica_MB1
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DON2_dromaea_SK1
DON3_dromaea_SK1
DON5_dromaea_SK1
DON1jv1_dromaea_SK1F
DON1jv2_dromaea_SK1F
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DMNS7_tristis_CO
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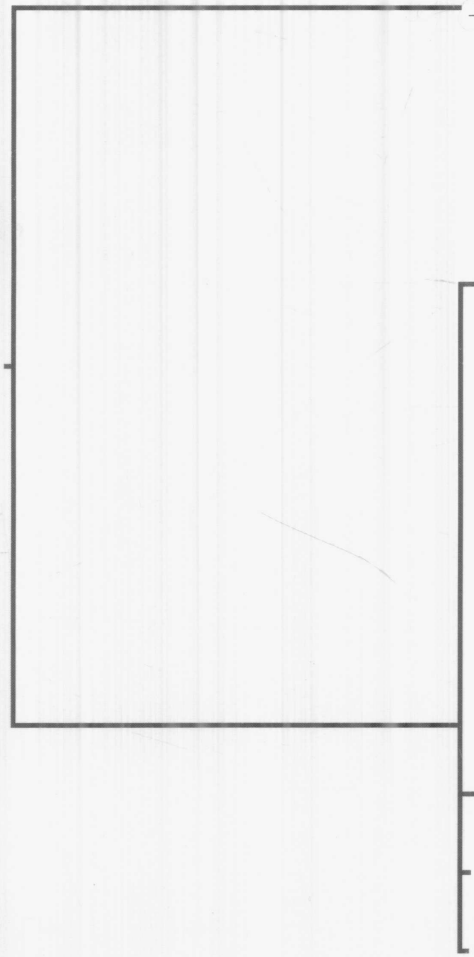
45656_prosaica_AK3

Figure 2.2 - Bayesian 90% majority rule consensus phylogram for species of the *Pardosa groenlandica* species complex and outgroup based on 480 bp of the mitochondrial NDI gene using a three partitioned GTR+I+G model for each codon position. Values above branches are posterior probabilities. The grey box highlights basal group, further explained in text. Grey specimens represent those which would be included in *P. groenlandica* based on morphological results of Chapter 1.



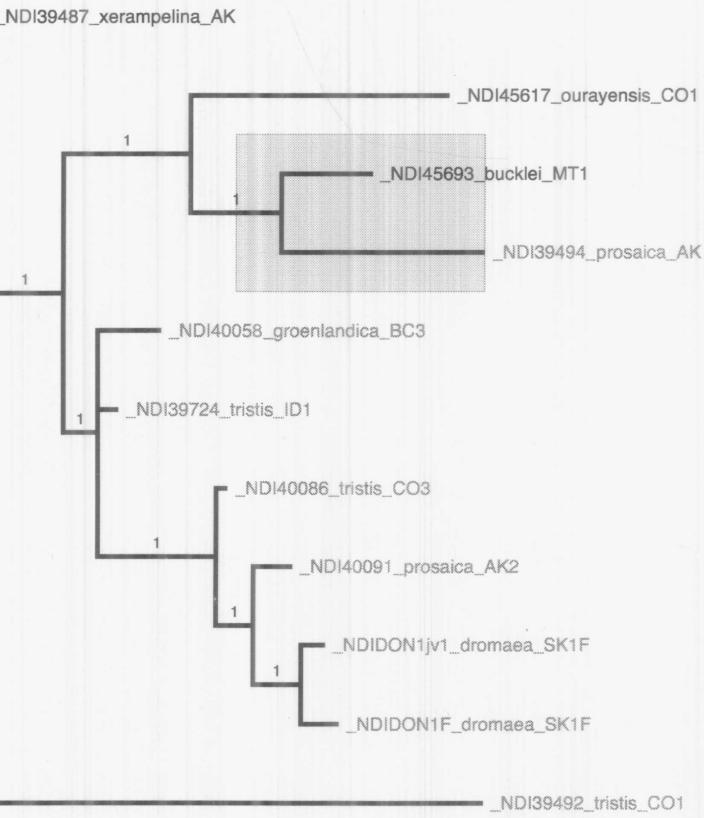

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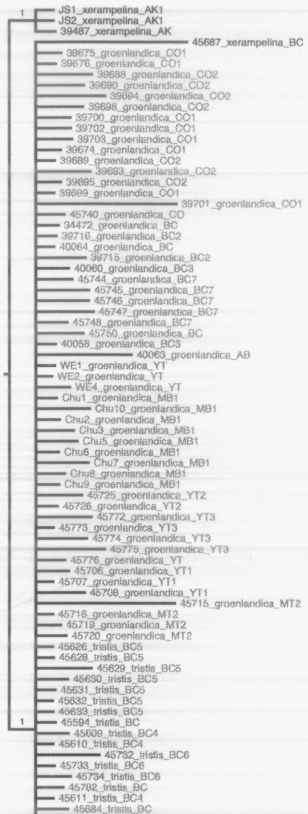
Figure 2.3 - Bayesian 90% majority rule consensus phylogram for species of the *Pardosa groenlandica* species complex and outgroup based on 456 bp of the nuclear internal transcribed spacer (ITS) region 1. The grey specimens represent those which would be included in *P. groenlandica* based on morphological results of Chapter 1.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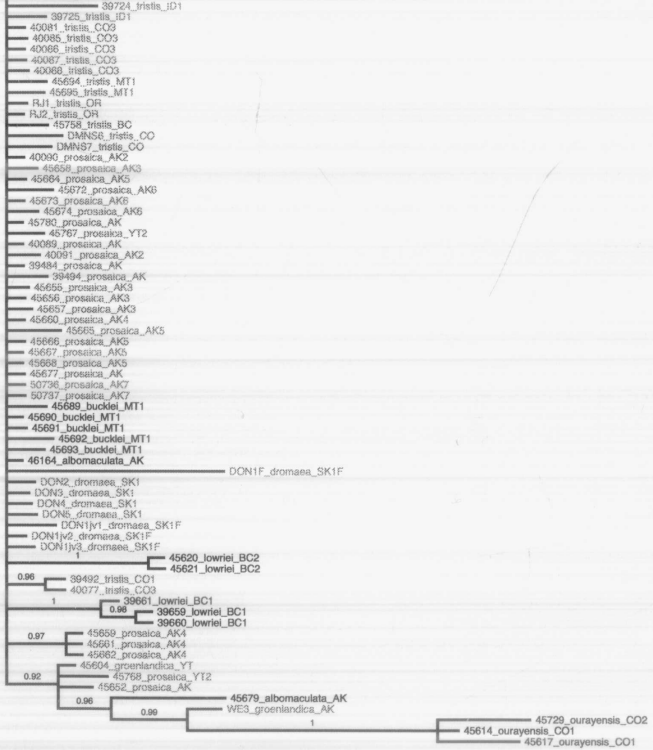
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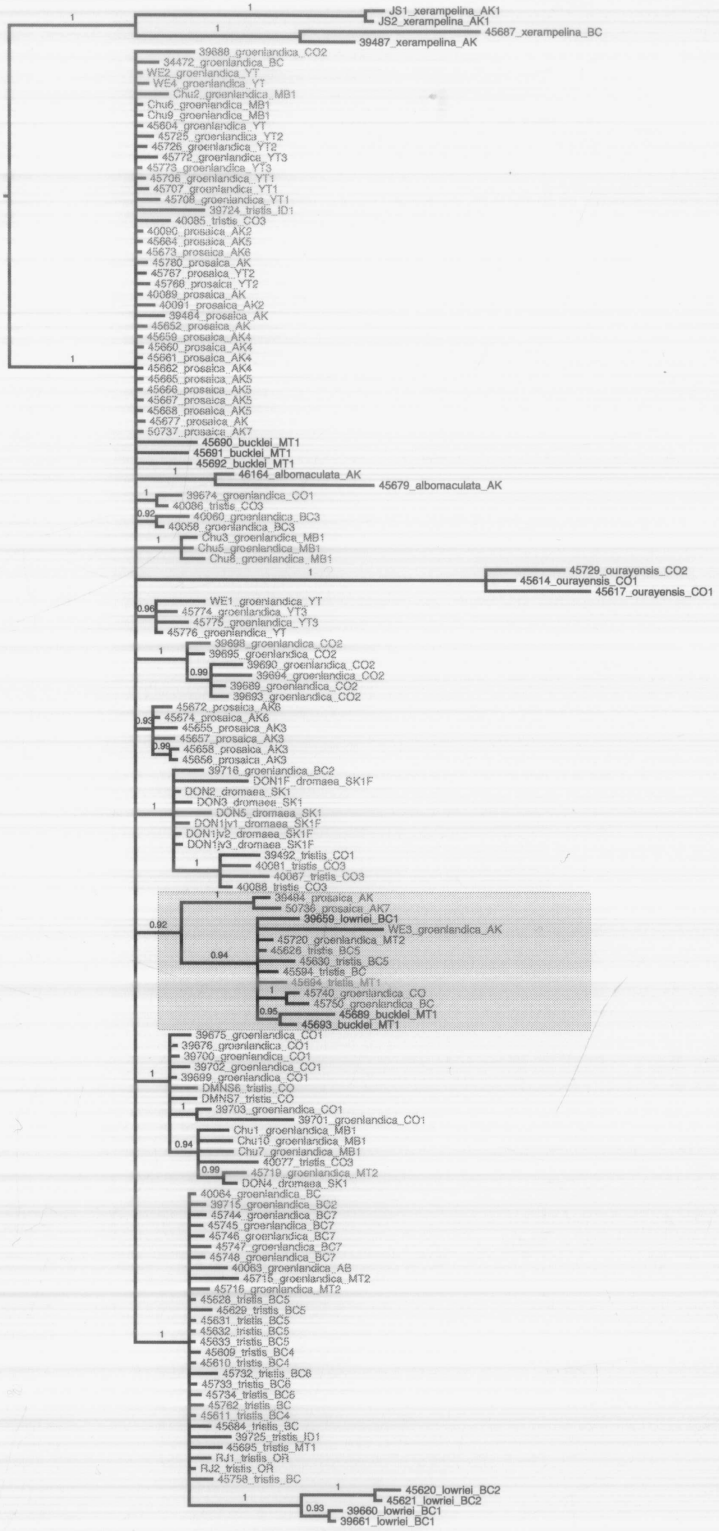
45614_ourayensis_CO1
45617_ourayensis_CO1

Figure 2.4 - Bayesian 90% majority rule consensus phylogram for species of the *Pardosa groenlandica* species complex and outgroup species using a four partition model (P4i) based on the COI (GTR+I+G for each codon position), ITS1, 5.8S, and ITS2 genes (HKY+I+G for all nuclear genes). Grey box highlights basal group, further explained in text. The grey specimens represent those which would be included in *P. groenlandica* based on morphological results of Chapter 1.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45780_prosaica_AK
45767_prosaica_YT2
45768_prosaica_YT2
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40058_groenlandica_BC3
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45614_ourayensis_CO1
45617_ourayensis_CO1
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RJ2_tristis_OR
45758_tristis_BC
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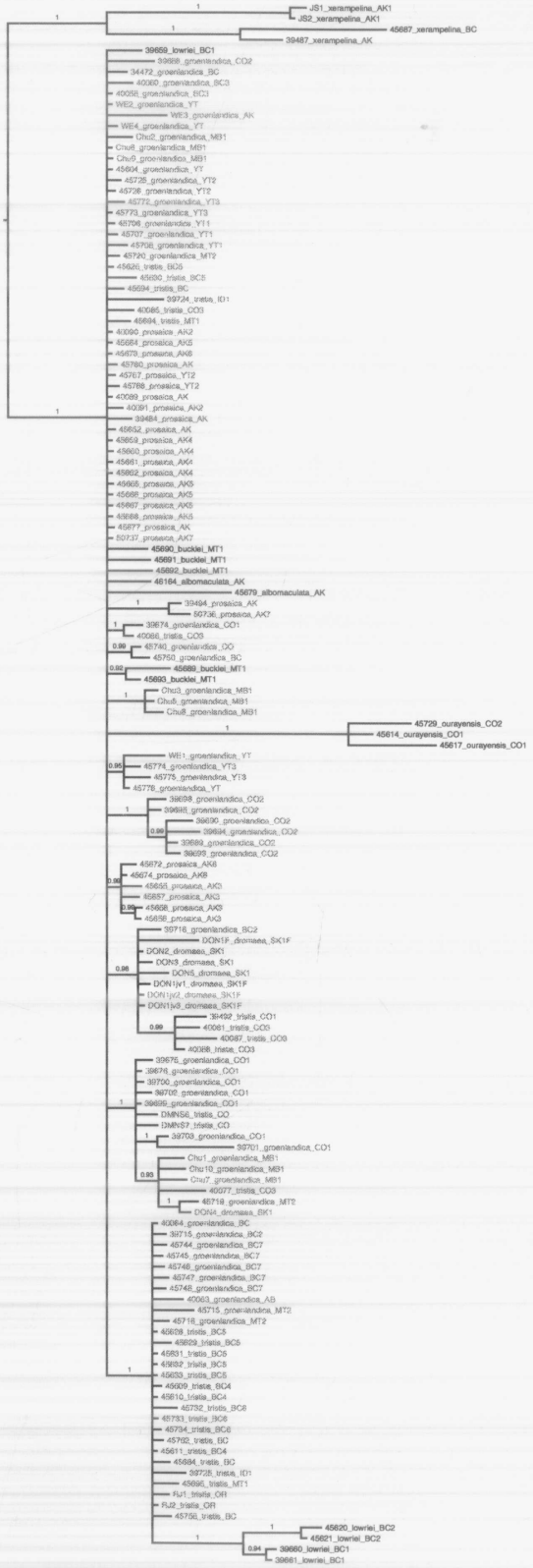
45621_lowriei_BC2
39660_lowriei_BC1
39661_lowriei_BC1

Figure 2.5 - Bayesian 90% majority rule consensus phylogram for species of the *Pardosa groenlandica* species complex and outgroup species using a five partition model (P5) based on the COI (GTR+I+G for each codon position), ITS1 (HKY+I+G), 5.8S, and ITS2 (K80) genes. The grey specimens represent those which would be included in *P. groenlandica* based on morphological results of Chapter 1.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40058_groenlandica_BC1
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WE3_groenlandica_AK
WE4_groenlandica_YT
Chu2_groenlandica_MB1
Chu8_groenlandica_MB1
Chu9_groenlandica_MB1
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45707_groenlandica_YT1
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45720_groenlandica_MT2
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45694_tristis_BC
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45684_tristis_MT1
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45684_prosaica_AK5
45679_prosaica_AK5
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45788_prosaica_YT2
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40091_prosaica_AK2
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Chu5_groenlandica_MB1
Chu8_groenlandica_MB1
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45617_ourayensis_CO1
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39660_lowriei_BC1
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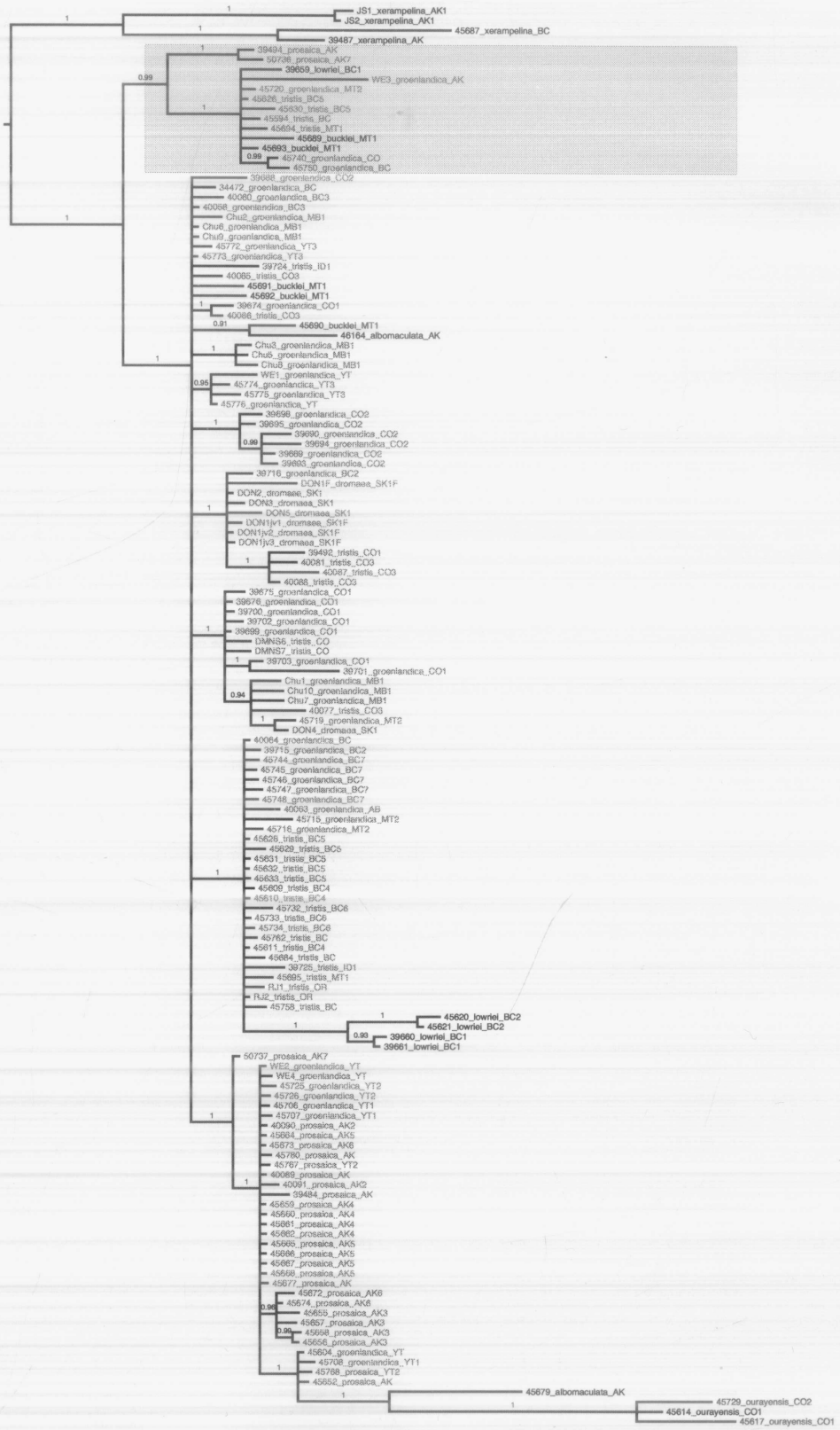
Figure 2.6 - Bayesian 90% majority rule consensus phylogram for species of the *Pardosa groenlandica* species complex and outgroup species using a six partition model (P6i) based on the COI (GTR+I+G for each codon position), ITS1 (HKY+I+G), 5.8S (K80), and ITS2 (F81) genes. The grey box highlights basal group, further explained in text. Grey specimens represent those which would be included in *P. groenlandica* based on morphological results of Chapter 1.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45693_bucklei_MT1
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45750_groenlandica_BC
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40056_groenlandica_BC3
Chu2_groenlandica_MB1
Chu6_groenlandica_MB1
Chu9_groenlandica_MB1
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45692_bucklei_MT1
39674_groenlandica_CO1
40086_tristis_CO3
45690_bucklei_MT1
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Chu5_groenlandica_MB1
Chu8_groenlandica_MB1
WE1_groenlandica_YT
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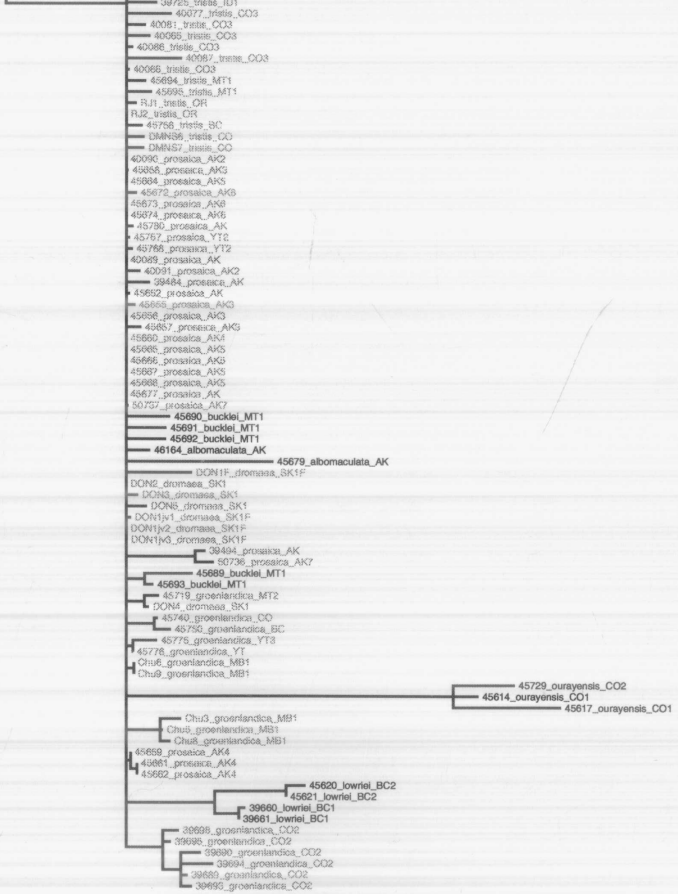
Figure 2.7 – Maximum likelihood bootstrap 70% consensus tree for species of the *Pardosa groenlandica* species complex and outgroup species based on 100 bootstrap replicates of the GTR+I+G model with a combined dataset of the COI, ITS1, 5.8S, and ITS2 genes. The grey specimens represent those which would be included in *P. groenlandica* based on morphological results of Chapter 1.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RJ2_tristis_OR
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DMNS7_tristis_CO
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DON3_dromaea_SK1
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45776_groenlandica_YT
Chu6_groenlandica_MB1
Chu9_groenlandica_MB1
45729_ourayensis_CO2
45614_ourayensis_CO1
45617_ourayensis_CO1
Chu3_groenlandica_MB1
Chu5_groenlandica_MB1
Chu8_groenlandica_MB1
45659_prosaica_AK4
45661_prosaica_AK4
45662_prosaica_AK4
45620_lowriei_BC2
45621_lowriei_BC2
39660_lowriei_BC1
39661_lowriei_BC1
39695_groenlandica_CO2
39696_groenlandica_CO2
39690_groenlandica_CO2
39694_groenlandica_CO2
39689_groenlandica_CO2
39693_groenlandica_CO2


JS1_xerampelina_AK1
JS2_xerampelina_AK1
45687_xerampelina_BC
39487_xerampelina_AK
39659_lowriei_BC1
39675_groenlandica_CO1
39678_groenlandica_CO1
39888_groenlandica_CO2
39700_groenlandica_CO1
39702_groenlandica_CO1
39703_groenlandica_CO1
39674_groenlandica_CO1
39696_groenlandica_CO1
39701_groenlandica_CO1
34472_groenlandica_BC
39716_groenlandica_BC2
40064_groenlandica_BC
39715_groenlandica_BC2
40060_groenlandica_BC3
45744_groenlandica_BC7
45749_groenlandica_BC7
45746_groenlandica_BC7
45747_groenlandica_BC7
45748_groenlandica_BC7
40058_groenlandica_BC3
40063_groenlandica_AB
WE1_groenlandica_YT
WE2_groenlandica_YT
WE3_groenlandica_AK
WE4_groenlandica_YT
Chu1_groenlandica_MB1
Chu10_groenlandica_MB1
Chu2_groenlandica_MB1
Chu7_groenlandica_MB1
45604_groenlandica_YT
45725_groenlandica_YT2
45726_groenlandica_YT2
45772_groenlandica_YT3
45773_groenlandica_YT3
45774_groenlandica_YT3
45706_groenlandica_YT1
45707_groenlandica_YT1
45708_groenlandica_YT1
45715_groenlandica_MT2
45716_groenlandica_MT2
45720_groenlandica_MT2
45626_tristis_BC5
45628_tristis_BC5
45629_tristis_BC5
45630_tristis_BC5
45631_tristis_BC5
45632_tristis_BC5
45633_tristis_BC5
45594_tristis_BC
45609_tristis_BC4
45610_tristis_BC4
45732_tristis_BC6
45733_tristis_BC6
45734_tristis_BC6
45782_tristis_BC
45611_tristis_BC4
45684_tristis_BC
39492_tristis_CO1
39724_tristis_sD1

Figure 2.8 - Neighbor joining analysis of the *Pardosa groenlandica* species complex using Kimura 2-parameter (K2P) distances of the barcode region of the COI gene. Grey box highlights basal group, further explained in text. The grey specimens represent those which would be included in *P. groenlandica* based on morphological results of Chapter 1.

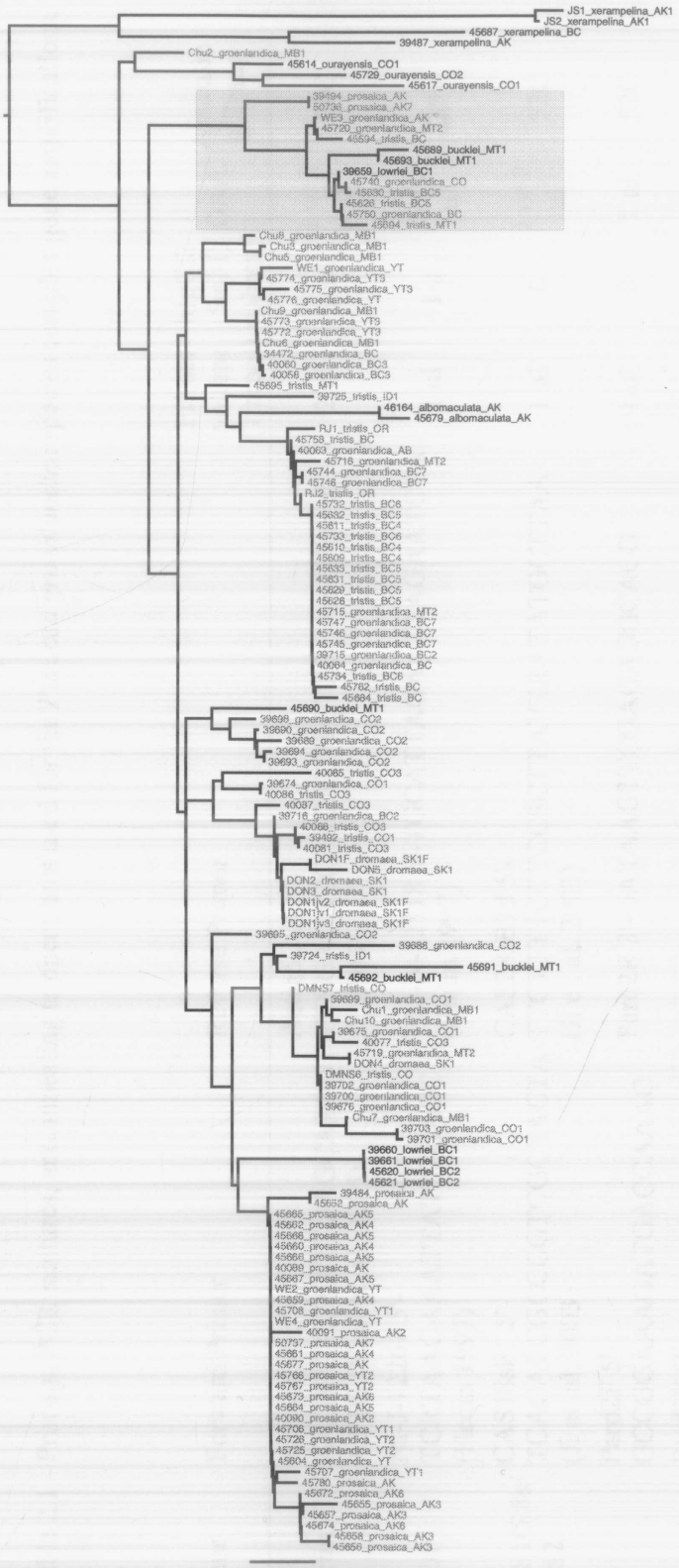
JS1_xerampelina_AK1
JS2_xerampelina_AK1
45687_xerampelina_BC
39487_xerampelina_AK
Chu2_groenlandica_MB1
45614_ourayensis_CO1
45729_ourayensis_CO2
45617_ourayensis_CO1
39494_prosaica_AK
50736_prosaica_AK7
WE3_groenlandica_AK
45720_groenlandica_MT2
45594_tristis_BC
45689_bucklei_MT1
45693_bucklei_MT1
39659_lowriei_BC1
45740_groenlandica_CO
45690_tristis_BC5
45626_tristis_BC5
45750_groenlandica_BC
45694_tristis_MT1
Chu8_groenlandica_MB1
Chu3_groenlandica_MB1
Chu5_groenlandica_MB1
WE1_groenlandica_YT
45774_groenlandica_YT5
45775_groenlandica_YT3
45776_groenlandica_YT
Chu9_groenlandica_MB1
45773_groenlandica_YT5
45772_groenlandica_YT3
Chu6_groenlandica_MB1
34472_groenlandica_BC
40060_groenlandica_BC3
40056_groenlandica_BC3
45695_tristis_MT1
39725_tristis_ID1
46164_albomaculata_AK
45679_albomaculata_AK
RJ1_tristis_OR
45758_tristis_BC
40063_groenlandica_AB
45716_groenlandica_MT2
45744_groenlandica_BC7
45748_groenlandica_BC7
RJ2_tristis_OR
45732_tristis_BC6
45632_tristis_BC5
45611_tristis_BC4
45733_tristis_BC6
45610_tristis_BC4
45609_tristis_BC4
45635_tristis_BC5
45631_tristis_BC5
45629_tristis_BC5
45628_tristis_BC5
45715_groenlandica_MT2
45747_groenlandica_BC7
45746_groenlandica_BC7
45745_groenlandica_BC7
39715_groenlandica_BC2
40064_groenlandica_BC
45734_tristis_BC6
45762_tristis_BC
45684_tristis_BC
45690_bucklei_MT1
39689_groenlandica_CO2
39690_groenlandica_CO2
39689_groenlandica_CO2
39694_groenlandica_CO2
39693_groenlandica_CO2
40085_tristis_CO3
39674_groenlandica_CO1
40086_tristis_CO3
40087_tristis_CO3
39718_groenlandica_BC2
40088_tristis_CO3
39492_tristis_CO1
40081_tristis_CO3
DON1F_dromaea_SK1F
DON5_dromaea_SK1
DON2_dromaea_SK1
DON3_dromaea_SK1
DON1jv2_dromaea_SK1F
DON1jv1_dromaea_SK1F
DON1jv3_dromaea_SK1F
39695_groenlandica_CO2
39688_groenlandica_CO2
39724_tristis_ID1
45691_bucklei_MT1
45692_bucklei_MT1
DMNS7_tristis_CO
39669_groenlandica_CO1
Chu1_groenlandica_MB1
Chu10_groenlandica_MB1
39675_groenlandica_CO1
40077_tristis_CO3
45719_groenlandica_MT2
DON4_dromaea_SK1
DMNS6_tristis_CO
39702_groenlandica_CO1
39700_groenlandica_CO1
39676_groenlandica_CO1
Chu7_groenlandica_MB1
39703_groenlandica_CO1
39701_groenlandica_CO1
39660_lowriei_BC1
39661_lowriei_BC1
45620_lowriei_BC2
45621_lowriei_BC2
39484_prosaica_AK
45652_prosaica_AK
45665_prosaica_AK5
45662_prosaica_AK4
45666_prosaica_AK5
45660_prosaica_AK4
45656_prosaica_AK5
40089_prosaica_AK
45667_prosaica_AK5
WE2_groenlandica_YT
40659_prosaica_AK4
45708_groenlandica_YT1
WE4_groenlandica_YT
40091_prosaica_AK2
50757_prosaica_AK7
45661_prosaica_AK4
45677_prosaica_AK
45768_prosaica_YT2
45767_prosaica_YT2
45673_prosaica_AK6
45664_prosaica_AK5
40090_prosaica_AK2
45706_groenlandica_YT1
45728_groenlandica_YT2
45725_groenlandica_YT2
45804_groenlandica_YT
45707_groenlandica_YT1
45780_prosaica_AK
45672_prosaica_AK6
45655_prosaica_AK3
45657_prosaica_AK3
45674_prosaica_AK6
45658_prosaica_AK3
45656_prosaica_AK3
0.0050

Table 2.1 - Primers, PCR mixtures, and annealing temperatures used for the *Pardosa groenlandica* species complex gene amplifications.

Gene	Forward Primer	Reverse Primer	Primer volume (1 nmol)	MgCl2 (5mM) volume	DNA	Annealing Temp
Actin5C	Actin5C-F 5'-AAGTATCCNATTGAGCATGGTATTG (Vink et. al. 2005)	Actin5C-R 5'-TTNGADATCCACATTTGTTGGAA (Vink et. al. 2005)	2 ul	1 ul	1 or 2 ul	54C
NDI	NI-J-12261 5'-TCRTAAGAAATTATTTGAGC (Hedin 1997)	TLI-N-12718 5'-TGCATTAGAATTAGAATCTA (Hedin 1997)	1 ul	1 ul	1 or 2 ul	54C
ITS1/5.8s/ ITS2	CAS18sF1 5'-TCACACCGCCCGTCGCTACTA (Ji et. al. 2003)	CAS28sB1d 5'-TTCTTTTCCTCCSCTTAYTRATATGCTTAA (Ji et. al. 2003)	1 ul	0	1 or 2 ul	52C
	Pard2F 5'-GCCGGAAAGATGACCAAAC	Pard2R 3'-GATAAGTTCGAGAGGCACG	1 ul	0	1 ul	52C
	Pard3F 5'-AAGTCGTAACAAGGTTTCCG	Pard2R 3'-GATAAGTTCGAGAGGCACG	1 ul	0	1 ul	51C
ITS2	ITS2F 5'-GATCGACACTTCGAACGA (Chang et. al. 2007)	ITS2R 5'-GCCGTTACTGAGGGAATCCTG (Chang et. al. 2007)	1 ul	0	1 ul	52C
COI	LCO-1490 5'-GGTCAACAAATCATAAAGATATTGG (Folmer et. al. 1994)	C1-N-2568 5'-GCTACAACATAATAAGTATCATG (Hedin & Maddison 2001)	2 ul	1 ul	4 ul	48C

Table 2.1 - Cont.

C1-J-1718-spider 5'-GGNGGATTTGGAAATTGRTTRGTTCC (Vink et. al. 2005)	C1-N-2776-spider 5'-GGATAATCAGAATANCGNCGAGG (Vink et. al. 2005)	2 ul	1 ul	4 ul	54C
LCO-1490 5'-GGTCAACAAATCATAAAGATATTGG (Folmer et. al. 1994)	C1-N-2776-spider 5'-GGATAATCAGAATANCGNCGAGG (Vink et. al. 2005)	2 ul	1 ul	4 ul	48C

Table 2.2 - Partition schemes for different Mr.Bayes analyses run on the *Pardosa groenlandica* species complex genetic datasets. Branch length correction follow recommendations of Marshall (2010) and Brown *et al.* (2010), further explained in text.

Partition Name	Number of partitions	Branch Length Correction	Model	Gene
P1	1	Yes	GTR+I+G	COI, ITS1, 5.8s, ITS2
P1i	1	No	GTR+I+G	COI, ITS1, 5.8s, ITS2
P2i	2	Yes	GTR+I+G	COI
			HKY+I+G	ITS1, 5.8s, ITS2
P2ii	2	Yes	HKY+I+G	COI 1st & 2nd codon pos., ITS1, 5.8s, ITS2
			GTR+I+G	COI 3rd codon pos.
P4i	4	Yes	GTR+I+G	COI 1st, 2nd, & 3rd codon pos.
			HWY+I+G	ITS1, 5.8s, ITS2
P4ii	4	Yes	GTR+I+G	COI
			HKY+I+G	ITS1
			K80	5.8s
			F81	ITS2
P5	5	Yes	GTR+I+G	COI 1st, 2nd, & 3rd codon pos.
			HKY+I+G	ITS1
			K80	5.8s & ITS2
P6	6	No	GTR+I+G	COI 1st, 2nd, & 3rd codon pos.
			HKY+I+G	ITS1
			K80	5.8s
			F81	ITS2
P6i	6	Yes	GTR+I+G	COI 1st, 2nd, & 3rd codon pos.
			HKY+I+G	ITS1
			K80	5.8s
			F81	ITS2

Table 2.3 - Locality codes for populations used in phylogenetic analysis.

Place	State	Local	Lat	Long
AK1	ALASKA	Fels Glacier	63.37400	-145.55000
AK2	ALASKA	Fairbanks Airport, Tanana River	64.79070	-147.88560
AK3	ALASKA	Tiekel River	61.32320	-145.31331
AK4	ALASKA	Prince William Sound	61.10731	-146.23408
AK5	ALASKA	Richardson Hwy, Lower Miller Creek	63.39400	-145.73235
AK6	ALASKA	Edgerton Hwy, Tolsina River	61.65215	-144.65489
AK7	ALASKA	Tok Cut Off	62.30120	-145.30555
BC1	BRITISH COLUMBIA	Toad River	58.82116	-125.01768
BC2	BRITISH COLUMBIA	Mobery Lake	55.81112	-121.70137
BC2 on P. lowriei samples	ALASKA	Haines, Chilkat River	59.43377	-136.23609
BC3	BRITISH COLUMBIA	Stikine River	58.04401	-129.95134
BC4	BRITISH COLUMBIA	Sorrento, Shuswap Lake	50.89293	-119.81934
BC5	BRITISH COLUMBIA	Kamloops. Kamloops Lake	50.76346	-120.80546
BC6	BRITISH COLUMBIA	70 Mile House, Green Lake	51.39094	-121.28377
BC7	BRITISH COLUMBIA	Scookumchuk, Kootenay River	49.91115	-115.73646
CO1	COLORADO	Mt. Evans	38.58382	-105.62623
COI* on P. ourayensis samples	COLORADO	El Paso Co. Pikes Peak	38.84590	-105.05195
CO2	COLORADO	Pike Peak	38.86401	-105.06943
CO2* on P. ourayensis samples	COLORADO	Larimer Co. Dixon Reservoir	40.55380	-105.14133
CO3	COLORADO	Green Mountain Reservoir, MacDonald Flats CG	39.84839	-106.23555

Table 2.3 – Cont.

ID1	IDAHO	Snake River	43.72296	-112.08754
MB1	MANITOBA	Churchill, bird cove	58.76370	-93.89700
MT1	MONTANA	Clark Canyon Reservoir	44.99837	-112.85577
MT2	MONTANA	Kalispel, Somers Beach	48.07571	-114.21905
SK1	SASKATCHAWAN	Saskatoon	52.07000	-106.38000
YT1	YUKON	Kluane Lake	61.00665	-138.43763
YT2	YUKON	White River	61.98742	-140.55829
YT3	YUKON	Teslin Lake	60.23202	-132.91196

Table 2.4 - Harmonic means, Bayes factors, split frequencies, and Effective Sample Sizes (ESS) for different partition schemes used on the combined dataset consisting of the COI, ITS1, 5.8S, and ITS2 genes.

Model	Combined harmonic mean	SE	Best harmonic mean	SE	Run that was best	Split Frequency	ESS values over 100, or parameters not over 100
p1	-7105.43	2.28	-7102.44	1.82	2	0.076	yes
p1i	-7273.21	1.54	-7252.46	4.62	2	0.089	A-G run1
p2i	-6920.7	5.66	-6904.6	2.82	1	0.015	no
p2ii	-7087.74	3.61	-7078.94	4.01	2	0.018	yes
p4i	-6622.36	2.2	-6614.07	2.12	2	0.073	a1, m1, m3 run1
p4ii	-6942.5	1.18	-6896.96	0.9	2	0.070	no
p5	-6608.09	0.86	-6602.68	3.19	2	0.100	a1 run2
p6	-6626.83	0.54	-6624.78	2	2	0.100	no
p6i	-6609.9	1.78	-6608.05	3.19	2	0.025	yes

Combined	B10
p2i/p1	184.74 strong
p2i/p2ii	167.04 strong
p4i/p2i	298.33 very strong
P4i/P4ii	320.14 very strong
p4i/p5	-14.28 positive
p4i/p6i	-12.46 positive
p5/p6i	1.81 bare mention
Best	
p2i/p1	197.84
p2i/p2ii	174.34
p4i/p2i	290.53
P4i/P4ii	282.89
p4i/p5	-11.39
p4i/p6i	-6.02
p5/p6i	5.37

Table 2.5 - Total genetic variation of the *Pardosa groenlandica* species complex and outgroup species using uncorrected *p*-distances.

	P. xerampelina	*P. ourayensis*	*P. lowriei*	*P. groenlandica*	*P. tristis*	*P. prosaica*	*P. bucklei*	*P. albomaculata*	*P. dromaea*
P. xerampelina	0.024	0.046	0.041	0.037	0.037	0.037	0.037	0.041	0.036
P. ourayensis	0.046	0.011	0.036	0.033	0.036	0.031	0.034	0.032	0.034
P. lowriei	0.041	0.036	0.013	0.018	0.017	0.017	0.021	0.024	0.020
P. groenlandica	0.037	0.033	0.018	0.011	0.013	0.011	0.015	0.020	0.011
P. tristis	0.037	0.036	0.017	0.013	0.012	0.014	0.016	0.021	0.013
P. prosaica	0.037	0.031	0.017	0.011	0.014	0.005	0.015	0.019	0.011
P. bucklei	0.037	0.034	0.021	0.015	0.016	0.015	0.016	0.022	0.015
P. albomaculata	0.041	0.032	0.024	0.020	0.021	0.019	0.022	0.013	0.022
P. dromaea	0.036	0.034	0.020	0.011	0.013	0.011	0.015	0.022	0.006

Chapter 3

Population genetics of *Pardosa groenlandica* and *Pardosa tristis* in Colorado

Abstract

Colorado is home to the type locations for several of the *Pardosa groenlandica* members. Populations from the type locations of two species, *P. groenlandica* (as *P. iracunda*) and *P. tristis,* were compared using morphometrics, phylogenetics, and an AMOVA analysis. Results find that while each population contained unique epiygnal morphologies, there was no molecular support for the morphologies. These results question the possibility of more than one species of the *Pardosa groenlandica* group occurring in Colorado.

Introduction

Colorado is home to the type locations for a great many pardosine spiders. Of the *Pardosa groenlandica* species complex, three species were described from the front range around Denver (Thorell 1872). *Pardosa iracunda* (=*P. groenlandica,* Dondale 1999) was described from the upper slopes of Pike's Peak, south of Denver at about 3,900 m. *Pardosa tristis* was described from Manitou Springs, located at the base of Pike's Peak, as well as Idaho Springs further north and west of Denver, and *P. indagatrix* (=*P. dromaea,* Thorell 1878) from Denver. All three species were originally described using few specimens in poor condition. Original delineations of the three species relied on characters of the epigyna, as both *P. tristis* and *P. indagatrix* were described from only females. Dondale's (1999) review found the palp

uninformative for delineation of these species, but he did describe epigynal, size, and distribution characters useful for species identification. These characters correspond to the *sensu strico* descriptions given in Dondale (1999) and the three median septum (MS) shapes described in Chapter 1. The individual species type locations are quite different in elevation and habitat. Thorell (1877) described the three species from a collection not made by himself. Therefore, any habitat information he received was second hand, and it's not clear how he used that information in his decision to erect these forms as species. Thorell's (1877) Colorado types have been lost, so it is impossible to verify his original descriptions.

Dondale (Dondale and Redner 1990, Dondale 1999) confronted the issue of lost types by attempting to collect neotypes from the type locations. He was able to collect neotypes of *P. iracunda* from Pike's Peak, but unfortunately he found the type locations of *P. tristis* and *P. dromaea* drastically altered by development. Because Idaho Springs and Manitou Springs habitats were altered by highway improvements, he had to move the type location of *P. tristis* to the nearest population he could locate, 3,900 m up on Mt. Evans, a habitat similar to Pike's Peak. Also, he was unable to locate any *P. dromaea* specimens from Denver proper, and he was forced to move the neotype location 16 km outside of Denver. Collecting trips in 2009 failed to find any specimens at the original type locations for *P. tristis* or *P. dromaea*, nor the neotype location of *P. dromaea*, as this area is currently being developed and the habitat is no longer suitable for the species.

Examination of specimens from throughout the range of these three species, as well as *P. prosaica,* found the morphology of the epigyna to be variable among specimens of the same population (Chapter 1). This resulted in a failure to correctly

separate specimens of the four species without knowing habitat and distribution information. The inability to distinguish these species resulted in a new species assemblage, in which *P. tristis, P. dromaea,* and *P. prosaica* were synonymized under *P. groenlandica.* These three species were found to be polyphyletic using molecular systematics; however, several other morphologically distinct species of the *groenlandica* complex were also found to be polyphyletic (Chapter 2). Further, these results did not provide evidence for or against either the currently recognized three species found in Colorado, or for the synonymy proposed in Chapter 1. The morphological differences of the four species proposed in Chapter 1 are comparable to the amount used for species delineation in *Pardosa* (Dondale and Redner 1990, Vogel 2004), which argues against the possibility of these all being one variable species.

However, these two species-assemblage hypotheses may be testable at the population level. For *P. tristis* to be valid, specimens collected from a population with different MS shapes would be sympatric species, and should show some level of molecular divergence. Furthermore, samples from the type locations for *P. iracunda* (=*P. groenlandica*) and *P. tristis,* Pike's Peak and Mt. Evans, should also show some level of molecular divergence. However, if the two type locations show extensive mixing, then these two populations may be the same species. A failure to identify more than one species from Colorado questions Thorell's (1877) original designations and Dondale's synonymy of *P. iracunda.*

Methods

Specimen collection techniques and sequencing were summarized earlier

(Chapters 1 and 2). In this study I used specimens from four populations in and around Colorado (Appendix 3). These populations were: the neotype location for *P. tristis*, Mt. Evans, 3,900 m (CO-1), the type location for *P iracunda*(=*groenlandica*), Pike's Peak, 3,900 m (CO-2), Green Mountain Reservoir, 2400 m, (a population of *P. tristis* similar in elevation to Idaho Springs, the original type location, CO-3), and a collection of *P. tristis* specimens collected at lower elevations (~1,000 m) outside of Colorado from the Great Basin and Central Rockies (Figure 3.1).

Specimens were assessed morphologically, measured for carapace length, carapace width and, if female, the p/q ratio of the epigyna was determined (Dondale 1999). To determine size differences among populations, a corrected carapace size was calculated by multiplying carapace length by width and then multiplying by 0.75 as a correction factor for the shape of the carapace in the rectangular area. The p/q ratio and carapace size were compared among populations and among high and low elevations using an ANOVA.

Specimens were analyzed phylogenetically using methods described in Chapter 2 for each gene using Bayesian methods to construct trees. *Pardosa ourayensis* was used as an outgroup species. Three different group schemes of population assemblages were analyzed using an AMOVA run in Arlequin v 3.1 (Excoffier *et al.* 2005) for the mtDNA and the rDNA. These were: four independent populations (4 groups), high elevations vs. low elevations (2 groups), and groups of individuals sharing similar MS shape for the two *sensu stricto* species vs. specimens with MS shapes not described as *sensu stricto* (4 groups). Because of the high number of parsimony-informative sites in both gene regions, the Tamura-Nei (TrN) distance metric was used for all distance analyses as that is the most complex distance metric

available in Arlequin and was the model selected by Modeltest for the phylogenetic analysis.

Results

Examination of specimens for each population found three populations with unique MS shapes. Inverted T-shaped median septa were found only at Mt. Evans (n=2), bottle-shaped median septa were only found at Pike's Peak (n=2), and a third MS shape, one in which the MS does not expand posteriorly and is somewhat "I"-shaped, was found only at Somers Beach, Montana (n=2). Five specimens from several populations had "A"-shaped median septa (Appendix 3). All other specimens had varying MS shapes mentioned previously (Chapter 1).

ANOVA analysis of the carapace size of the specimens found a significant difference among the four populations (P=0.0053; d.f.=3, 40; F=4.917), with the high-elevation populations being smaller (P=0.0001; d.f.=45; t=4.23). Differences in the measurements of the epigyna p/q ratio among populations was found to be insignificant (P=0.61; d.f.=3, 25; F=0.6180).

Phylogenetic results were similar to those obtained earlier for COI and ITS1 with the entire 144-specimen dataset (Chapter 2, Figures 2.1 and 2.3). The mtDNA showed a loose population clade structure, but there was also extensive polyphyly among populations. The rDNA data were largely uninformative, with no population structure identified even when viewed using a Neighbor Joining Tree with TrN distances (Figure 3.2).

Average corrected COI distances between the outgroup, *P. ourayensis*, and the

ingroup was 0.034, where as the average among-population distance was 0.012. The Mt. Evans (CO-1) population showed the smallest amount of variation at the population level, 0.003. The Pike's Peak (CO-2), Green Mountain Reservoir (CO-3), and Great Basin populations were in a similar range, 0.01, 0.014, and0.016 respectively. Average rDNA distances between the outgroup species and ingroup populations was 0.038. Average within-population variation and variation between populations was 0.006 (Table 3.1).

AMOVA results of different group schemes for both mtDNA and rDNA showed high amounts of variation at the individual level. The COI data showed an increase in variation at the population level when each population was treated independently, however that structuring was not carried over in the rDNA (Table 3.2). Φst values were relatively higher when specimens were organized by populations rather than epigynal characters. Morphological differences among specimens and populations were not molecularly supported.

Discussion

Results from this study located morphological characters, carapace size and MS shape, that, as population-level characters, were not supported molecularly. At the population level these results contrast with Chang *et al.*'s (2007) investigation of *Pardosa astrigera* Koch 1878 in China, which has two morphological polymorphisms in the shape of the embolus. Their results found similar amounts of variation in mitochondrial and nuclear DNA to those found in this study (0.1-1.8% for COI, and 0.7% for ITS2, using uncorrected *p*-distances); however, they were able to identify

nuclear haplotypes, based on insertions in the ITS2 region, that corresponded with the two embolus shapes. These two phenotypes have never been considered separate species. Chang *et al.* concluded they were conspecific but suggestive of past incomplete divergence within the species.

Data from my study also failed to show strong support for geographic structure among the populations sampled. The population genetics studies of Muster and Berekdonk (2006) and Muster *et al.* (2009) examined a species complex of five European *Pardosa* species; however, the five species were treated as a single metapopulation. Their studies looked at several populations dispersed over a geographic range similar in size to population subset to my study (Appendix C), with about 1,100 km between the two most distant populations. The earlier study (Muster and Berekdonk 2006) used the mitochondrial gene NDI to examine southerly gene flow across the European landscape after recent glacial episodes. Their results found strong support for geographic structure among populations, with two southern clades and one northern clade. Their following study (Muster *et al.* 2009) added the nuclear genes ITS1, 5.8S, and ITS2 treated collectively as one rDNA gene. Phylogenetically, their rDNA results did not show the same geographic structure as their NDI results, but AMOVA and coalescent simulations results did. They did mention that the NDI results contradicted the valid five species, but they did not expand beyond this and included no morphological information on any of the species or populations or how those species were identified. None of their results presented identifications given to specimens; rather all specimens were treated as if they belonged to a single species in all of their analyses.

Taxonomically these results imply that previous assumptions of several

different species occupying the mountains of Colorado are not supported (Thorell 1877, Dondale 1999). The phylogenetic, AMOVA, Φst, and morphological results indicate that there is a single metapopulation of a single species occupying the mountains of Colorado northward and into the Great Basin/Central Rockies area. However, these results are unable to identify which species that is. It appears that Dondale's (1999) synonymy of *P. iracunda* with *P. groenlandica* may be in error as a more likely synonym would be with *P. tristis.* However, these data are unable to determine if *P. tristis* is a synonym of *P. groenlandica* as well.

Conclusion

These three chapters provide a thorough look at the existing taxonomy of the *Pardosa groenlandica* species complex. But in the end the results are largely inconclusive. Morphologically, there is support for four species (Chapter 1) with open questions about how much variation there is within a species and where that variation is geographically located. Phylogenetically, the mitochondrial results are mixed, with several distinct geographic clades that show no distinct morphological characters. For example, the clade of British Columbia specimens is strongly supported, but it lacks any corresponding morphological characters that identify those specimens. Contrasting those results are those of *P. bucklei*, which is polypheletic in all molecular analyses, yet clearly shows morphological identification characters. These contrasting results indicate that there are no clear molecular boundaries to species limits in this group. Results from examining the Colorado specimens at a population level showed strong support that all of the populations examined constitute a single

population. This questions the current assumption that *P. groenlandica* extends south along the Rockies, or alternatively, presents evidence for the synonymy of *P. tristis* with *P. groenlandica.* These results also found that although there were MS shapes distinct to three of the populations, the molecular analysis failed to find any molecular correlation.

Speciation is a process, and not all species show large interspecific genetic distances or reciprocal monophyly. These results fail to answer the original taxonomic question of whether the currently recognized seven species of the *groenlandica* species complex are indeed separate species. Rather, these results raise many more questions about the members of the species complex and its phylogeographic history.

It is important to clarify goals prior to a study, and to understand limitations of a dataset. One of the advantages of morphological assessments is that specimens from an entire species' range can often be acquired. Even though this study produced a large molecular dataset, ~1800 bp for 144 specimens, it failed to test several of the species hypotheses because of missing sample locations and small sample sizes of several species. This further emphasizes a limitation of molecular studies to inform taxonomic decisions, in which samples for populations of interest sometimes cannot be obtained.

More importantly, these results question how we have been taxonomically defining species of the genus *Pardosa.* Traditionally, taxonomy of the genus has been based on slight morphological differences in the genitalia. This study shows how species with clear morphological differences in the genitalia may not correlate with underlying structure in neutral genetic markers.

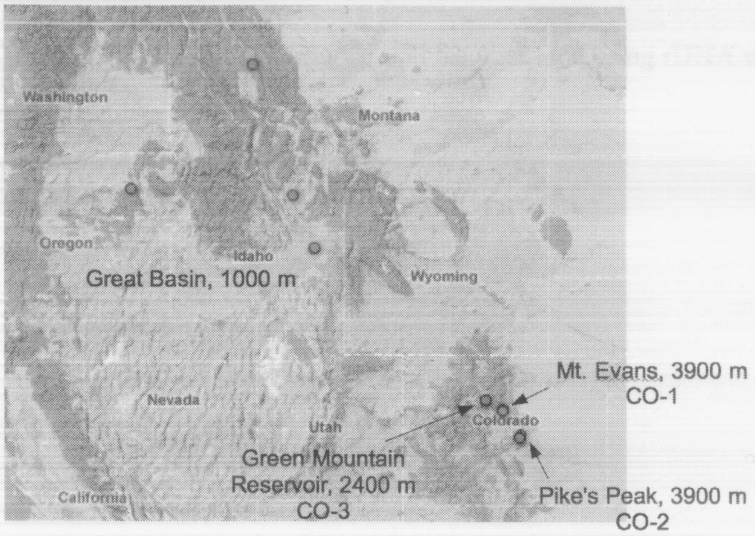

Figure 3.1 - Population locations for subset of Colorado specimens of *Pardosa groenlandica* and *P. tristis*.

Figure 3.2 - Neighbor joining analysis of *Pardosa groenlandica* and *P. tristis* specimens from Colorado and the Great Basin/Central Rockies area using rDNA with Tamura-Nei (TrN) distances.

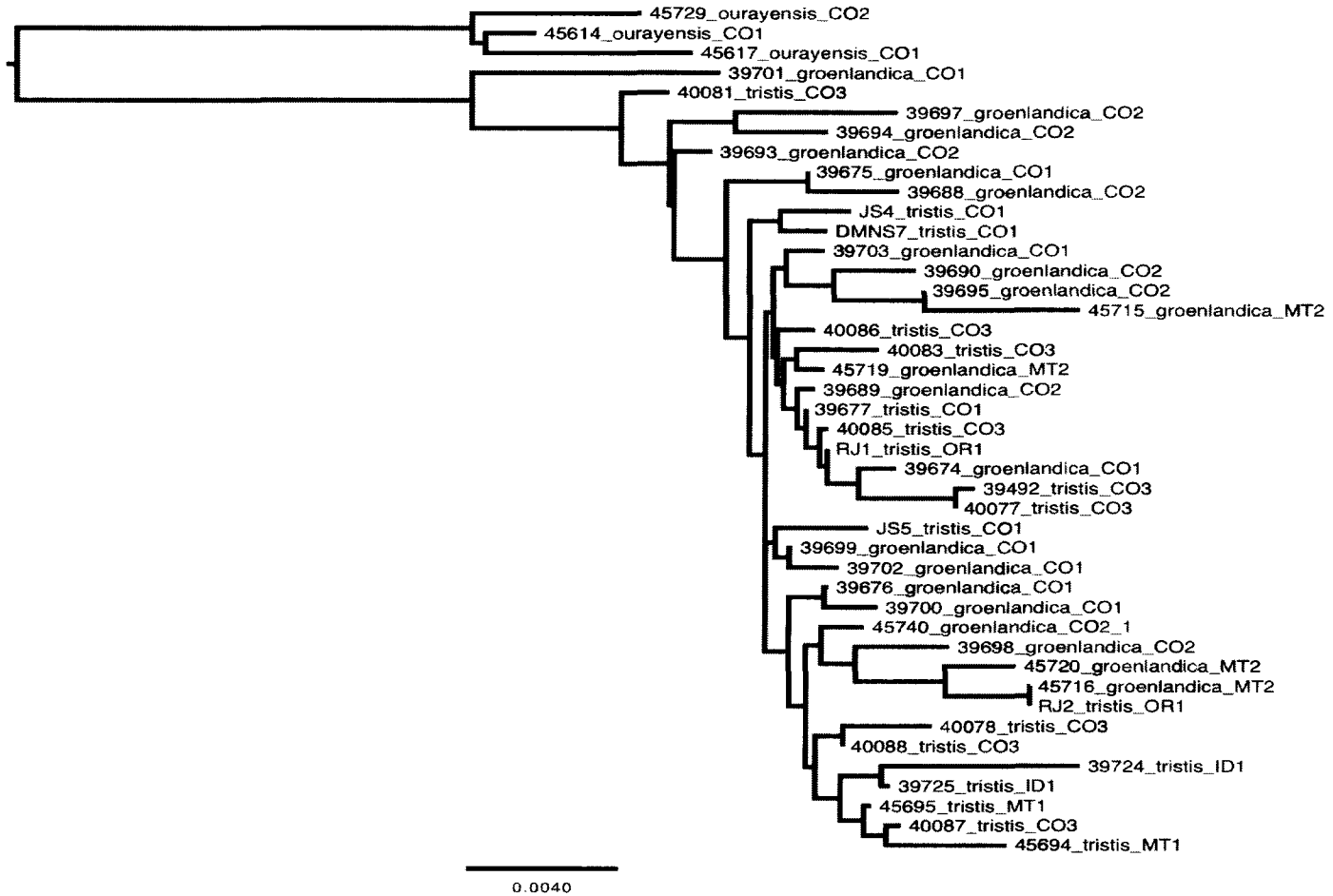
45729_ourayensis_CO2
45614_ourayensis_CO1
45617_ourayensis_CO1
39701_groenlandica_CO1
40081_tristis_CO3
39697_groenlandica_CO2
39694_groenlandica_CO2
39693_groenlandica_CO2
39675_groenlandica_CO1
39688_groenlandica_CO2
JS4_tristis_CO1
DMNS7_tristis_CO1
39703_groenlandica_CO1
39690_groenlandica_CO2
39695_groenlandica_CO2
45715_groenlandica_MT2
40086_tristis_CO3
40083_tristis_CO3
45719_groenlandica_MT2
39689_groenlandica_CO2
39677_tristis_CO1
40085_tristis_CO3
RJ1_tristis_OR1
39674_groenlandica_CO1
39492_tristis_CO3
40077_tristis_CO3
JS5_tristis_CO1
39699_groenlandica_CO1
39702_groenlandica_CO1
39676_groenlandica_CO1
39700_groenlandica_CO1
45740_groenlandica_CO2_1
39698_groenlandica_CO2
45720_groenlandica_MT2
45716_groenlandica_MT2
RJ2_tristis_OR1
40078_tristis_CO3
40088_tristis_CO3
39724_tristis_ID1
39725_tristis_ID1
45695_tristis_MT1
40087_tristis_CO3
45694_tristis_MT1
0.0040

Table 3.1 - Distance matrix of *Pardosa groenlandica* and *P. tristis* specimens from Colorado and the Great Basin/Central Rockies area using Tamura-Nei distances.

COI

Population	CO-1	CO-2	CO-3	Other	Out
CO1	0.00381				
CO2	0.01591	0.01060			
CO3	0.01406	0.01778	0.01430		
Other	0.01633	0.01951	0.01937	0.01648	
Out	0.03192	0.03452	0.03510	0.03458	0.01327

Elevation	Hi	Low
Hi	0.01059	
Low	0.01652	0.01754

ITS

Population	CO-1	CO-2	CO-3	Other	Out
CO1	0.00565				
CO2	0.00697	0.00660			
CO3	0.00589	0.00655	0.00545		
Other	0.00811	0.00810	0.00762	0.00781	
Out	0.03812	0.03718	0.03783	0.03924	0.00770

Elevation	Hi	Low
Hi	0.00649	
Low	0.00721	0.00719

Table 3.2 - Results of AMOVA analysis of *Pardosa groenlandica* and *P. tristis* specimens from Colorado and the Great Basin/Central Rockies area comparing several different population structure schemes for the ITS1, 5.8S and ITS2 (combined) and COI genes.

COI	4 Populations	Hi vs Low Elevation	Epigyna Shape
Between Population	27.13	5.24	13.08
Between Individuals within Populations	13.65	34.83	6.27
Among Individuals	59.21	59.92	80.65
Φst	0.41	0.4	0.19
rbDNA			
Between Population	5.52	2.39	-10.46
Between Individuals within Populations	9.16	12.58	15.78
Among Individuals	85.32	85.03	94.69
Φst	0.14	0.15	0.05

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Appendix 1

Label data of specimens used in this study. DMNS = Denver Museum of Nature and Science Arachnid Collection, CNC = Voucher specimens from the Canadian National Collection, UAM = University of Alaska Museum Insect Collection, Chu = University of Ontario, Guelph, RJ = Personal collection of R. J. Adams, Fresno, California, Don = Personal collection of D. J. Buckle, Saskatoon, Saskatchewan WE = Personal collection of S. Wise-Eagle, Wrangell, Alaska, JS = Personal collection of the author. * = used in phylogenetic analyses, ** = used in both phylogenetic and population analyses, E# = number of COI extractions above 1, P# = number of COI PCR amplifications above 1.

Specimen Number		Species Name	Sex	State/Province	Locality	Latitude	Longitude	Elevation (m)
UAM100045679	**	*Pardosa albomaculata* Emerton, 1885	1 female	ALASKA	Denali park, E of entrance	63.74251	-148.86361	
UAM100046164	**	*Pardosa albomaculata* Emerton, 1885	1 female	ALASKA	Kenai	59.85380	-150.67350	
CNC		*Pardosa bucklei* Kronestedt, 1975	2 male 3 female	OREGON	Fish Lake, Steen Mtns.	42.38894	-122.32860	1413
CNC		*Pardosa bucklei* Kronestedt, 1975	2 male 3 female	ARIZONA	Mormon Lake	34.90835	-111.46320	2177

Appendix 1 – Cont.

UAM100045689	**	*Pardosa bucklei* Kronestedt, 1975	1 male	MONTANA	Clark Canyon Res.	44.99837	-112.85577	1678
UAM100045690	**	*Pardosa bucklei* Kronestedt, 1975	1 male	MONTANA	Clark Canyon Res.	44.99837	-112.85577	1678
UAM100045691	**	*Pardosa bucklei* Kronestedt, 1975	1 male	MONTANA	Clark Canyon Res.	44.99837	-112.85577	1678
UAM100045692	**	*Pardosa bucklei* Kronestedt, 1975	1 female	MONTANA	Clark Canyon Res.	44.99837	-112.85577	1678
UAM100045693	** P2	*Pardosa bucklei* Kronestedt, 1975	1 female	MONTANA	Clark Canyon Res.	44.99837	-112.85577	1678
CNC		*Pardosa dromea* (Thorell, 1878)	1 male 1 female	ALBERTA	16 km west of Lethbridge	49.69349	-112.84184	893
CNC		*Pardosa dromea* (Thorell, 1878)	2 female	ALBERTA	Hussar	51.04194	-112.68305	908
CNC		*Pardosa dromea* (Thorell, 1878)	2 male 2 female	ALBERTA	Woolford Prov. Park	49.27041	-113.21203	1136
Don1 F	** E2 P3	*Pardosa dromea* (Thorell, 1878)	1 female	SASKATCHAWAN	Saskatoon	52.07000	-106.38000	
Don1 jv1	** P2	*Pardosa dromea* (Thorell, 1878)	1 jv	SASKATCHAWAN	Saskatoon	52.07000	-106.38000	
Don1 jv2	**	*Pardosa dromea* (Thorell, 1878)	1 jv	SASKATCHAWAN	Saskatoon	52.07000	-106.38000	
Don1 jv3	**	*Pardosa dromea* (Thorell, 1878)	1 jv	SASKATCHAWAN	Saskatoon	52.07000	-106.38000	

Appendix 1 – Cont.

Don2	**		*Pardosa dromea* (Thorell, 1878)
Don3	**		*Pardosa dromea* (Thorell, 1878)
Don4	**		*Pardosa dromea* (Thorell, 1878)
Don5	**		*Pardosa dromea* (Thorell, 1878)
Chu 1	**		*Pardosa groenlandica* (Thorell, 1872)
Chu 10	**	P2	*Pardosa groenlandica* (Thorell, 1872)
Chu 2	**		*Pardosa groenlandica* (Thorell, 1872)
Chu 3	**		*Pardosa groenlandica* (Thorell, 1872)
Chu 4			*Pardosa groenlandica* (Thorell, 1872)
Chu 5	**		*Pardosa groenlandica* (Thorell, 1872)
Chu 6	**		*Pardosa groenlandica* (Thorell, 1872)
Chu 7	**		*Pardosa groenlandica* (Thorell, 1872)

1 jv	SASKATCHAWAN	Saskatoon	52.07000	-106.38000	
1 jv	SASKATCHAWAN	Saskatoon	52.07000	-106.38000	
1 jv	SASKATCHAWAN	Saskatoon	52.07000	-106.38000	
1 jv	SASKATCHAWAN	Saskatoon	52.07000	-106.38000	
1 female	MANITOBA	Churchill, bird cove	58.76370	-93.89700	0
1 female	MANITOBA	Churchill, bird cove	58.76370	-93.89700	0
1 female	MANITOBA	Churchill, bird cove	58.76370	-93.89700	0
1 male	MANITOBA	Churchill, bird cove	58.76370	-93.89700	0
1 male	MANITOBA	Churchill, bird cove	58.76370	-93.89700	0
1 female	MANITOBA	Churchill, bird cove	58.76370	-93.89700	0
1 female	MANITOBA	Churchill, bird cove	58.76370	-93.89700	0
1 male	MANITOBA	Churchill, bird cove	58.76370	-93.89700	0

Appendix 1 – Cont.

Chu 8	**	*Pardosa groenlandica* (Thorell, 1872)	1 male	MANITOBA	Churchill, bird cove	58.76370	-93.89700	0
Chu 9	**	*Pardosa groenlandica* (Thorell, 1872)	1 male	MANITOBA	Churchill, bird cove	58.76370	-93.89700	0
CNC		*Pardosa groenlandica* (Thorell, 1872)	1 male	BRITISH COLUMBIA	Summit Lake, mi 372 Alaska Hwy	58.65000	-124.63333	1388
CNC		*Pardosa groenlandica* (Thorell, 1872)	1 male 1 female	NEWFOUNDLAND	Gros Morne National Park	49.61403	-57.71849	436
CNC		*Pardosa groenlandica* (Thorell, 1872)	2 female	BRITISH COLUMBIA	Sparwood	49.73170	-114.88762	1131
CNC		*Pardosa groenlandica* (Thorell, 1872)	5 male 5 female	GREENLAND	Sondestrom Air Base	67.01055	-50.70916	51
JS3		*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO	Mt. Evans	38.58382	-105.62623	
JS4		*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO	Mt. Evans	38.58382	-105.62623	
JS5		*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO	Mt. Evans	38.58382	-105.62623	
UAM100034472	* P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	BRITISH COLUMBIA	Stikine R.	58.04401	-129.95134	674
UAM100039674	**	*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO	Mt. Evans	38.58382	-105.62623	3947
UAM100039675	** P2	*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO	Mt. Evans	38.58382	-105.62623	3947

Appendix 1 – Cont.

UAM100039676	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039677			*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039688	**		*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO
UAM100039689	**		*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039690	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039693	**		*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039694	**		*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039695	**		*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039697			*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039698	**		*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO
UAM100039699	**		*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO
UAM100039700	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO

Mt. Evans	38.58382	-105.62623 3947
Mt. Evans	38.58382	-105.62623 3947
Pike's Peak	38.86401	-105.06943 3931
Pike's Peak	38.86401	-105.06943 3931
Pike's Peak	38.86401	-105.06943 3931
Pike's Peak	38.86401	-105.06943 3931
Pike's Peak	38.86401	-105.06943 3931
Pike's Peak	38.86401	-105.06943 3931
Pike's Peak	38.86401	-105.06943 3931
Pike's Peak	38.86401	-105.06943 3931
Mt. Evans	39.58631	-105.64441 4250
Mt. Evans	39.58631	-105.64441 4250

Appendix 1 – Cont.

UAM100039701	**		*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039702	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039703	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100040058	**		*Pardosa groenlandica* (Thorell, 1872)	1 female	BRITISH COLUMBIA
UAM100040059			*Pardosa groenlandica* (Thorell, 1872)	1 female	BRITISH COLUMBIA
UAM100040060	**		*Pardosa groenlandica* (Thorell, 1872)	1 female	BRITISH COLUMBIA
UAM100040062			*Pardosa groenlandica* (Thorell, 1872)	1 male	BRITISH COLUMBIA
UAM100040063	*		*Pardosa groenlandica* (Thorell, 1872)	1 male	ALBERTA
UAM100040064	*		*Pardosa groenlandica* (Thorell, 1872)	1 female	BRITISH COLUMBIA
UAM100045604	*	P3	*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
UAM100045706	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
UAM100045707	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON

Mt. Evans	39.58631	-105.64441	4250
Mt. Evans	39.58631	-105.64441	4250
Mt. Evans	39.58631	-105.64441	4250
Stikine R.	58.04401	-129.95134	674
Stikine R.	58.04401	-129.95134	674
Stikine R.	58.04401	-129.95134	674
Alaska Hwy	59.93333	-131.75595	800
Spray Lakes	50.99804	-115.36872	1684
Hwy 16	53.72630	-121.14411	710
Snag Creek	62.47652	-140.86710	638
Kluane Lake	61.00665	-138.43763	800
Kluane Lake	61.00665	-138.43763	800

Appendix 1 – Cont.

UAM100045708	**	*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
UAM100045709		*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
UAM100045715	** P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	MONTANA
UAM100045716	**	*Pardosa groenlandica* (Thorell, 1872)	1 female	MONTANA
UAM100045717		*Pardosa groenlandica* (Thorell, 1872)	1 female	MONTANA
UAM100045718		*Pardosa groenlandica* (Thorell, 1872)	1 female	MONTANA
UAM100045719	**	*Pardosa groenlandica* (Thorell, 1872)	1 female	MONTANA
UAM100045720	** P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	MONTANA
UAM100045725	** P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
UAM100045726	**	*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
UAM100045739		*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100045740	*	*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO

Kluane Lake	61.00665	-138.43763 800
Kluane Lake	61.00665	-138.43763 800
Kalispel, Somers Beach	48.07571	-114.21905 883
Kalispel, Somers Beach	48.07571	-114.21905 883
Kalispel, Somers Beach	48.07571	-114.21905 883
Kalispel, Somers Beach	48.07571	-114.21905 883
Kalispel, Somers Beach	48.07571	-114.21905 883
Kalispel, Somers Beach	48.07571	-114.21905 883
White River	61.98742	-140.55829 715
White River	61.98742	-140.55829 715
El Paso Co., Crystal Lake	38.91974	-105.02908 2812
El Paso Co., Crystal Lake	38.91974	-105.02908 2812

Appendix 1 – Cont.

UAM100045744	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 male	BRITISH COLUMBIA
UAM100045745	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 male	BRITISH COLUMBIA
UAM100045746	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 male	BRITISH COLUMBIA
UAM100045747	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	BRITISH COLUMBIA
UAM100045748	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	BRITISH COLUMBIA
UAM100045750	*	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	BRITISH COLUMBIA
UAM100045772	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
UAM100045773	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
UAM100045774	**		*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
UAM100045775	**	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
UAM100045776	*	P2	*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
UAM100045777			*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON

Scookumchuk, Kootenay R.	49.91115	-115.73646	767
Scookumchuk, Kootenay R.	49.91115	-115.73646	767
Scookumchuk, Kootenay R.	49.91115	-115.73646	767
Scookumchuk, Kootenay R.	49.91115	-115.73646	767
Scookumchuk, Kootenay R.	49.91115	-115.73646	767
Buckinghorse R.	57.38614	-122.85170	1000
Teslin Lake	60.23202	-132.91196	703
Teslin Lake	60.23202	-132.91196	703
Teslin Lake	60.23202	-132.91196	703
Teslin Lake	60.23202	-132.91196	703
Watson Lake, Liard R.	60.05012	-128.90300	621
Watson Lake, Liard R.	60.05012	-128.90300	621

Appendix 1 – Cont.

WE1	*		*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
WE2	*		*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
WE3	*		*Pardosa groenlandica* (Thorell, 1872)	1 female	ALASKA
WE4	*		*Pardosa groenlandica* (Thorell, 1872)	1 female	YUKON
UAM100039659	**	E2 P3	*Pardosa lowriei* Kronestedt, 1975	1 female	BRITISH COLUMBIA
UAM100039660	**		*Pardosa lowriei* Kronestedt, 1975	1 female	BRITISH COLUMBIA
UAM100039661	**		*Pardosa lowriei* Kronestedt, 1975	1 male	BRITISH COLUMBIA
UAM100045620	**		*Pardosa lowriei* Kronestedt, 1975	1 female	ALASKA
UAM100045621	**		*Pardosa lowriei* Kronestedt, 1975	1 female	ALASKA
UAM100045622			*Pardosa lowriei* Kronestedt, 1975	1 female	ALASKA
UAM100045614	**		*Pardosa ourayensis* Gertsch, 1933	1 female	COLORADO

Frog Creek, Dempster Hwy	67.37970	-134.15200
North Fork Pass, Ogilive Mtns	64.57823	-138.26060
American Creek Bridge, Taylor Hwy	64.70040	-141.31498
40 Mile River, nr Yukon Riv.	64.42179	-140.56794
Toad River	58.82116	-125.01768 705
Toad River	58.82116	-125.01768 705
Toad River	58.82116	-125.01768 705
Haines, Chilkat R.	59.43377	-136.23609 160
Haines, Chilkat R.	59.43377	-136.23609 160
Haines, Chilkat R.	59.43377	-136.23609 160
El Paso Co. Pikes Peak	38.84590	-105.05195 4058

Appendix 1 – Cont.

UAM100045617	** P2	*Pardosa ourayensis* Gertsch, 1933	1 female	COLORADO
UAM100045729	**	*Pardosa ourayensis* Gertsch, 1933	1 female	COLORADO
CNC		*Pardosa prosaica* Chamberlin & Ivie, 1947	5 male 11 female	YUKON
UAM100039484	*	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100039493		*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100039494	*	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100040089	*	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 male	ALASKA
UAM100040090	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100040091	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA

El Paso Co. Pikes Peak	38.84590	-105.05195	4058
Larimer Co. Dizon Res.	40.55380	-105.14133	1565
Tagish Lake	60.26143	-134.29606	668
Chena River S P, Between bridges 2 & 3	64.92951	-146.29580	253
Chatanika River, Mile 11 Elliot Hwy	65.08481	-147.72590	180
Chena River S P, Between bridges 2 & 3	64.92951	-146.29580	253
Chena River S P, Between bridges 2 & 3	64.92951	-146.29580	253
Fairbanks Airport, Tanana River	64.79070	-147.88560	130
Fairbanks Airport, Tanana River	64.79070	-147.88560	130

Appendix 1 – Cont.

UAM100045651		*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female w/babies	ALASKA
UAM100045652	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045655	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045656	** P2	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045657	** P2	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045658	** E2 P4	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045659	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045660	** P3	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA

Salcha, Tanana R.	64.34012	-146.86501	234
Salcha, Tanana R.	64.34012	-146.86501	234
Tiekel River	61.32320	-145.31331	394
Tiekel River	61.32320	-145.31331	394
Tiekel River	61.32320	-145.31331	394
Tiekel River	61.32320	-145.31331	394
Prince William Sound	61.10731	-146.23408	1
Prince William Sound	61.10731	-146.23408	1

Appendix 1 – Cont.

UAM100045661	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045662	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045664	** P2	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 male	ALASKA
UAM100045665	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045666	** P2	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045667	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045668	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045672	** P3	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA

Prince William Sound	61.10731	-146.23408	1
Prince William Sound	61.10731	-146.23408	1
Richardson Hwy, Lower Miller Creek	63.39400	-145.73235	777
Richardson Hwy, Lower Miller Creek	63.39400	-145.73235	777
Richardson Hwy, Lower Miller Creek	63.39400	-145.73235	777
Richardson Hwy, Lower Miller Creek	63.39400	-145.73235	777
Richardson Hwy, Lower Miller Creek	63.39400	-145.73235	777
Edgerton Hwy, Tolsina R.	61.65215	-144.65489	203

Appendix 1 – Cont.

UAM100045673	** P3	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045674	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045677	*	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045767	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	YUKON
UAM100045768	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	YUKON
UAM100045779		*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100045780	*	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA
UAM100050736	**	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female	ALASKA

Edgerton Hwy, Tolsina R.	61.65215	-144.65489	203
Edgerton Hwy, Tolsina R.	61.65215	-144.65489	203
Richardson Hwy, by Trims Creek	63.44728	-145.80861	709
Cracker Creek	60.81489	-136.84964	645
Cracker Creek	60.81489	-136.84964	645
Chatanika River, Mile 11 Elliot Hwy	65.08481	-147.72590	180
Chatanika River, Mile 11 Elliot Hwy	65.08481	-147.72590	180
Tok Cut Off	62.30120	-145.30555	447

Appendix 1 – Cont.

UAM100050737	**	P2	*Pardosa prosaica* Chamberlin & Ivie, 1947	1 female ALASKA
CNC			*Pardosa tristis* (Thorell, 1877)	2 male 2 female BRITISH COLUMBIA
CNC			*Pardosa tristis* (Thorell, 1877)	3 male 2 female BRITISH COLUMBIA
DMNS 1			*Pardosa tristis* (Thorell, 1877)	1 female COLORADO
DMNS 2			*Pardosa tristis* (Thorell, 1877)	1 female COLORADO
DMNS 3		P2	*Pardosa tristis* (Thorell, 1877)	1 female COLORADO
DMNS 4			*Pardosa tristis* (Thorell, 1877)	1 female COLORADO
DMNS 5			*Pardosa tristis* (Thorell, 1877)	1 female COLORADO
DMNS 6	**	P2	*Pardosa tristis* (Thorell, 1877)	1 male COLORADO
DMNS 7	**	P2	*Pardosa tristis* (Thorell, 1877)	1 female COLORADO
DMNS 8			*Pardosa tristis* (Thorell, 1877)	1 female COLORADO

Tok Cut Off	62.30120	-145.30555 447
McBride	53.30416	-120.16138 722
Takla Landing	55.48333	-125.96666 735
Mt. Evans	39.58341	-105.62613 3565
Mt. Evans	39.58341	-105.62613 3565
Mt. Evans	39.58341	-105.62613 3565
Four Mile Creek	39.20794	-106.16747 3879
Four Mile Creek	39.20794	-106.16747 3879
Four Mile Creek	39.20794	-106.16747 3879
Mt. Evans	39.59630	-105.64269 3920
Mt. Evans	39.59630	-105.64269 3920

Appendix 1 – Cont.

RJ 1	**		*Pardosa tristis* (Thorell, 1877)	1 male	OREGON
RJ 2	**		*Pardosa tristis* (Thorell, 1877)	1 female	OREGON
UAM100039489		P2	*Pardosa tristis* (Thorell, 1877)	1 female	COLORADO
UAM100039492	**	P2	*Pardosa tristis* (Thorell, 1877)	1 male	COLORADO
UAM100039714			*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100039715	**		*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100039716	**		*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100039724	**	P2	*Pardosa tristis* (Thorell, 1877)	1 female	IDAHO
UAM100039725	**		*Pardosa tristis* (Thorell, 1877)	1 female	IDAHO
UAM100040077	**		*Pardosa tristis* (Thorell, 1877)	1 female	COLORADO

Scroll Cart C.G.	45.15790	-118.37990	1554
Scroll Cart C.G.	45.15790	-118.37990	1554
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Mobery Lake	55.81112	-121.70137	688
Mobery Lake	55.81112	-121.70137	688
Mobery Lake	55.81112	-121.70137	688
Snake River	43.72296	-112.08754	1447
Snake River	43.72296	-112.08754	1447
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402

Appendix 1 – Cont.

UAM100040078		*Pardosa tristis* (Thorell, 1877)	1 male	COLORADO
UAM100040080		*Pardosa tristis* (Thorell, 1877)	1 female	COLORADO
UAM100040081	**	*Pardosa tristis* (Thorell, 1877)	1 female	COLORADO
UAM100040082		*Pardosa tristis* (Thorell, 1877)	1 female	COLORADO
UAM100040083		*Pardosa tristis* (Thorell, 1877)	1 female	COLORADO
UAM100040084	P2	*Pardosa tristis* (Thorell, 1877)	1 female	COLORADO
UAM100040085	**	*Pardosa tristis* (Thorell, 1877)	1 female	COLORADO
UAM100040086	**	*Pardosa tristis* (Thorell, 1877)	1 male	COLORADO

Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402

Appendix 1 – Cont.

UAM100040087	**		*Pardosa tristis* (Thorell, 1877)	1 male	COLORADO
UAM100040088	**	P2	*Pardosa tristis* (Thorell, 1877)	1 male	COLORADO
UAM100045594	*	P2	*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045595			*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045609	**		*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045610	**		*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045611	**		*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045626	**	E2 P3	*Pardosa tristis* (Thorell, 1877)	1 male	BRITISH COLUMBIA
UAM100045627			*Pardosa tristis* (Thorell, 1877)	1 male	BRITISH COLUMBIA
UAM100045628	**	P2	*Pardosa tristis* (Thorell, 1877)	1 male	BRITISH COLUMBIA
UAM100045629	**	P2	*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA

Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Williams Lake	52.12025	-122.05962	558
Williams Lake	52.12025	-122.05962	558
Sorrento, Shuswap Lake	50.89293	-119.81934	342
Sorrento, Shuswap Lake	50.89293	-119.81934	342
Sorrento, Shuswap Lake	50.89293	-119.81934	342
Kamloops. Kamloops Lake	50.76346	-120.80546	328
Kamloops. Kamloops Lake	50.76346	-120.80546	328
Kamloops. Kamloops Lake	50.76346	-120.80546	328
Kamloops. Kamloops Lake	50.76346	-120.80546	328

Appendix 1 – Cont.

UAM100045630	**	*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045631	**	*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045632	**	*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045633	** P2	*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045683		*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045684	*	*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045685		*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045694	**	*Pardosa tristis* (Thorell, 1877)	1 male	MONTANA
UAM100045695	** P2	*Pardosa tristis* (Thorell, 1877)	1 female	MONTANA
UAM100045732	** P2	*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045733	** P2	*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045734	**	*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA

Kamloops. Kamloops Lake	50.76346	-120.80546	328
Kamloops. Kamloops Lake	50.76346	-120.80546	328
Kamloops. Kamloops Lake	50.76346	-120.80546	328
Kamloops. Kamloops Lake	50.76346	-120.80546	328
Revelstoke, Columbia R.	51.00566	-118.22803	423
Revelstoke, Columbia R.	51.00566	-118.22803	423
Revelstoke, Columbia R.	51.00566	-118.22803	423
Clark Canyon Res.	44.99837	-112.85577	1678
Clark Canyon Res.	44.99837	-112.85577	1678
70 Mile House, Green Lake	51.39094	-121.28377	1070
70 Mile House, Green Lake	51.39094	-121.28377	1070
70 Mile House, Green Lake	51.39094	-121.28377	1070

Appendix 1 – Cont.

UAM100045757			*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045758	*		*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045759			*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045760			*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045761			*Pardosa tristis* (Thorell, 1877)	1 female	BRITISH COLUMBIA
UAM100045762	*	P3	*Pardosa tristis* (Thorell, 1877)	1 male	BRITISH COLUMBIA
UAM100045763			*Pardosa tristis* (Thorell, 1877)	1 male	BRITISH COLUMBIA
JS1	**		*Pardosa xerampelina* (Keyserling, 1877)	1 jv	ALASKA
JS2	**		*Pardosa xerampelina* (Keyserling, 1877)	1 jv	ALASKA
UAM100039159			*Pardosa xerampelina* (Keyserling, 1877)	1 male	ALASKA
UAM100039365			*Pardosa xerampelina* (Keyserling, 1877)	1 male	COLORADO
UAM100039366			*Pardosa xerampelina* (Keyserling, 1877)	3 male 1 jv	COLORADO

Prince George, Fraiser R.	53.92778	-122.77873	558
Prince George, Fraiser R.	53.92778	-122.77873	558
Prince George, Fraiser R.	53.92778	-122.77873	558
Prince George, Fraiser R.	53.92778	-122.77873	558
Prince George, Fraiser R.	53.92778	-122.77873	558
Prince George, Fraiser R.	53.92778	-122.77873	558
Prince George, Fraiser R.	53.92778	-122.77873	558
Fels Glacier	63.37400	-145.55000	
Fels Glacier	63.37400	-145.55000	
Fairbanks, 531a Narwhale Trail	64.88281	-147.78452	166
Jefferson Co. Cub Creek Park	39.61063	-105.32660	2231
Jefferson Co. Cub Creek Park	39.61770	-105.32636	2179

Appendix 1 – Cont.

UAM100039487	*	*Pardosa xerampelina* (Keyserling, 1877)	1 female	ALASKA
UAM100040074		*Pardosa xerampelina* (Keyserling, 1877)	1 female	ALASKA
UAM100045607		*Pardosa xerampelina* (Keyserling, 1877)	2 femaqle	YUKON
UAM100045676		*Pardosa xerampelina* (Keyserling, 1877)	1 male 1 female	ALASKA
UAM100045687	**	*Pardosa xerampelina* (Keyserling, 1877)	1 female	BRITISH COLUMBIA
UAM100045711		*Pardosa xerampelina* (Keyserling, 1877)	1 female	BRITISH COLUMBIA
UAM100045743		*Pardosa xerampelina* (Keyserling, 1877)	1 female	BRITISH COLUMBIA
UAM100045751		*Pardosa xerampelina* (Keyserling, 1877)	1 female	BRITISH COLUMBIA
UAM100045770		*Pardosa xerampelina* (Keyserling, 1877)	1 female	YUKON
UAM100045782A	P2	*Pardosa xerampelina* (Keyserling, 1877)	1 female	ALASKA

Chena River S P, Between bridges 2 & 3	64.92294	-146.31738	243
Chena River S P, Between bridges 2 & 3	64.92294	-146.31738	243
Snag Creek	62.47652	-140.86710	638
Mi 141 Gulkana R.	62.45748	-145.42201	549
Revelstoke, Columbia R.	51.00566	-118.22803	423
Pine pass, Pine R.	55.44720	-122.72329	780
Scookumchuk, Kootenay R.	49.91115	-115.73646	767
Buckinghorse R.	57.38614	-122.85170	1000
Teslin Lake	60.23202	-132.91196	703
Chatanika River, Mile 11 Elliot Hwy	65.08481	-147.72590	180

Appendix 1 – Cont.

UAM100045782B	*Pardosa xerampelina* (Keyserling, 1877)	1 female	ALASKA	Chatanika River, Mile 11 Elliot Hwy	65.08481	-147.72590	180

Appendix 2

Additional information on sequencing results including information on the steps taken to verify genetic results.

NDI

Thirteen specimens sequenced, fragment length 480 bp, of which 38 bp were parsimony informative. The variable sites were predominantly located in the 2nd (40%) and 3rd (46%) codon positions. Base frequencies: A=36.1%, C=12.6%, G=8.5%, T=42.4%, 8.5% success rate of sequencing (13:151). This nucleotide composition is not uncommon for mitochondria in invertebrates and similar ratios have been reported (Vink and Patterson 2003).

Use of NDI for *Pardosa* species has occurred before (Muster and Berendonk 2006), but in my study annealing of the primer NI-J-12261 (Hedin 1997) became troublesome, causing failure in most PCR reactions. This resulted in a heavy-sized smear on the gels. Sequencing of the smears worked for the desired region when ExTaq (www.takara-bio.us) was used but always failed using the GoTaq Master Mix. It is unclear what the differences in the Taq mixtures may be.

COI

One hundred seventy two specimens sequenced, fragment length 1120 bp, of which 134 bp were parsimony informative. The variable sites were predominantly located in the 3rd codon position (49.4%), with the other two positions being equally split (25.3%). Base frequencies: A=26.4%, C=12.1%, G=18.5%, T=42.9%.

An initial phylogenetic analysis involving 51 specimens included a basal clade

consisting of multiple species of the species complex. Because of this peculiar grouping, examination of the possibility of numts was done. To look for ambiguities between reads and sequences, 38% (56 specimens) had PCR amplification and or DNA extraction replicated two or three times (Appendix A Koutroumpa *et al.* 2008). Additionally, various primer pairs were tried (LCO-1490 and C1-N-2776-spider, LCO-1490 and C1-N-2568, C1-J-1718-spider and C1-N-2776-spider, C1-J-1718-spider and C1-N-2568) to look for changes in sequences obtained from different primer sets (Moulton *et al.* 2010). No nonfunctional copies were located which could be identified as a numt. Additionally ambiguities between reads were rare, 4% of reads, and not reproducible and likely sequencing error.

The possibility of *Wolbachia* being present was examined. Seven specimens from the basal clade had PCR amplification using the *Wolbachia*-specific primer pair wsp81F-TGGTCCAATAAGTGATGAAGAAAC and wsp691R-AAAAATTAAACGCTACTCCA following the protocol and PCR mixture described by Rowley *et al.* (2004). All results testing for *Wolbachia* were negative. Also the results of the NDI gene also support this basal group (Figure 9). Because all of these attempts to identify the presence of numts failed it is assumed no numts are responsible for this basal group.

Actin5C

Six specimens sequenced, fragment length 900 bp, of which 63 bp were parsimony informative. Base frequencies: A=28.2%, C=20.6%, G =19.6%, T=31.0%, 4% success rate of sequencing (6:135).

The Actin5C gene initially produced promising results with bands at about

1000 bp. However, a faint double band in the 200 bp region was consistent. Upon sequencing, the 200 bp region was all that could be obtained. This band consisted of fragments of the far 3' end of the gene, in the 760-950 bp region. Attempts were made to physically remove the 1000 bp band from the gels and amplify or sequence it, but again the 200 bp region would dominate the results. By comparing the few clean entire gene reads it appears there is a similarity in the *Pardosa* sequences which allows the forward primer to attach near the rear of the gene. The Actin gene was not used for phylogenetic analysis.

ITS1, 5.8S, and ITS2

One hundred sixty six specimen sequenced for ITS1, 5.8S and ITS2. ITS1 fragment length 456 bp, of which 50 were parsimony informative. Base frequencies: A=23.8%, C=27.8%, G=28.0%, T=18.7%, which are similar in composition to those reported for nuclear rRNA genes (Hendrixson and Bond 2005).

Ribosomal subunit 5.8S fragment length 109 bp, of which none were parsimony informative. Base frequencies: A=23.9%, C=23.8%, G=29.1%, T=23.2%. Figure 2.B shows the nucleotide composition compared to those published for *Enoplognatha ovata* (Clerck 1757) (Tan *et al.* 1999). Only one haplotype was found for all *Pardosa* specimens examined in this study. ITS2 fragment length 190 bp, of which one was parsimony informative, base frequencies: A=19.8%, C=29.8%, G=33.0%, T=15.0%. This amplified region corresponds to helixes I, II and III. No changes in helix III were found.

Initial amplification of the ITS1, 5.8S, and ITS2 region was done using the CAS18sF1 and CAS28sB1d (Ji *et al.* 2003) primer pair. Initial results were

inconsistent with very messy chromatograms. Clear sequences were obtained for both *Pardosa* and several fungal species. Fungal sequences were obtained from specimens UAM100039492, UAM100040086 from Green Mountain Reservoir, CO and UAM100039494 from the Chena River, AK. Searches using GenBank BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the Fungal Metagenomics Search Engine (www.borealfungi.uaf.edu/) resulted in the same result for all sequences, *Golovinomyces cichoracearum* (D.C.), a species of Aspen blight. Results from BOLD (Bar Code of Life Data Systems; www.boldsystems.org) were less productive, providing best matches with several unidentified genera. The extraction and PCR amplification dates of each specimen were different, implying that the blight's spores were on the spider's legs and were extracted at the same time as the spider DNA. The amount of DNA extracted from a single leg is small, ~10 *nmol/ul*, but it is still surprising that the fungal DNA would have such an impact. Fungal contamination of spider DNA is not new and has been reported with this primer set previously (Muster *et al.* 2009; Vink *et al.* 2008).

From the clean spider ITS sequences I developed *Pardosa* specific primers Pard2F, Pard3F, and Pard2R (Table 3). These allowed for the amplification of about 2/3 of the ITS1 gene, all of the 5.8S, and about 2/3 of the ITS2 gene, totaling about 800 bp.

Comparing ITS2 data from this study to data for the entire ITS2 gene for the Asian *Pardosa astrigera* Koch 1878 (Chang *et al.* 2007) showed only a single diagnostic base change (site 131 in the 3' direct, G-T) for the 190 bp region of overlap. Secondary structure comparison of the *Pardosa astrigera* agreed with the expected ITS2 conformation and includes all four helices (Coleman 2009). Coleman

mentions changes in helix III may result in infertility and helix IV the most useful in species comparisons. No changes were found in helix III of specimens used, and no part of helix IV was amplified. In contrast, comparison of my ITS2 data with that of the *Pardosa saltuaria* group (Muster *et al.* 2009) showed considerable changes requiring several gaps for alignment and an inability to align *P. saltuaria* ITS2 data with expected ITS2 confirmations.

Figure 2.B - Comparison of the entire 5.8S gene sequence for *Pardosa* (Pard) and *Enoplognatha ovata* (Eno).

```
Pard-CAGTGGATCACTCGGCTCACGGGTCGATGAAGAACGCAGCCAGCTGCGAG
Eno -.................................................

Pard-ACTTGGTGTGAATTGCAGGACACATTGAGCACTGATTTTTCGAACGCACA
Eno -...-.............................................

Pard-TTGCGGTCT
Eno -......C..
```

Appendix 3

Specimens information of the subset used for the population genetics analysis of Colorado *Pardosa groenlandica* and *P. tristis.*

Specimen Number	Species Name	Sex	State/Provence	Locality	Latitude	Longitude	Elevation (m)	MS shape
DMNS 1	*Pardosa tristis* (Thorell)	1 female	COLORADO	Mt. Evans	39.58341	-105.62613	3565	Inverted T
DMNS 2	*Pardosa tristis* (Thorell)	1 female	COLORADO	Mt. Evans	39.58341	-105.62613	3565	
DMNS 3	*Pardosa tristis* (Thorell)	1 female	COLORADO	Mt. Evans	39.58341	-105.62613	3565	
DMNS 7	*Pardosa tristis* (Thorell)	1 female	COLORADO	Mt. Evans	39.59630	-105.64269	3920	
JS4	*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO	Mt. Evans	39.58382	-105.62623	3920	
JS5	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO	Mt. Evans	39.58382	-105.62623	3920	
UAM100039674	*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO	Mt. Evans	39.58382	-105.62623	3947	

Appendix 3 – Cont.

UAM100039675	*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO	Mt. Evans
UAM100039676	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO	Mt. Evans
UAM100039677	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO	Mt. Evans
UAM100039699	*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO	Mt. Evans
UAM100039700	*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO	Mt. Evans
UAM100039701	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO	Mt. Evans

39.58382	-105.62623	3947	
39.58382	-105.62623	3947	
39.58382	-105.62623	3947	
39.58631	-105.64441	4250	
39.58631	-105.64441	4250	
39.58631	-105.64441	4250	Inverted T

Appendix 3 – Cont.

UAM100039702	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039703	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039688	*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO
UAM100039689	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039690	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039693	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO

Mt. Evans	39.58631	-105.64441	4250	
Mt. Evans	39.58631	-105.64441	4250	A
Pike's Peak	38.86401	-105.06943	3931	
Pike's Peak	38.86401	-105.06943	3931	
Pike's Peak	38.86401	-105.06943	3931	Bottle
Pike's Peak	38.86401	-105.06943	3931	Bottle

Appendix 3 – Cont.

UAM100039694	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039695	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039697	*Pardosa groenlandica* (Thorell, 1872)	1 female	COLORADO
UAM100039698	*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO
UAM100045740	*Pardosa groenlandica* (Thorell, 1872)	1 male	COLORADO
UAM100039489	*Pardosa tristis* (Thorell, 1877)	1 female	COLORADO

Pike's Peak	38.86401	-105.06943	3931
Pike's Peak	38.86401	-105.06943	3931
Pike's Peak	38.86401	-105.06943	3931
Pike's Peak	38.86401	-105.06943	3931
El Paso Co., Crystal Lake	38.91974	-105.02908	2812
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402

Appendix 3 – Cont.

UAM100039492	*Pardosa tristis* (Thorell, 1877) 1 male	COLORADO
UAM100040077	*Pardosa tristis* (Thorell, 1877) 1 female	COLORADO
UAM100040078	*Pardosa tristis* (Thorell, 1877) 1 male	COLORADO
UAM100040081	*Pardosa tristis* (Thorell, 1877) 1 female	COLORADO
UAM100040082	*Pardosa tristis* (Thorell, 1877) 1 female	COLORADO

Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402	
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402	A
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402	
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402	
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402	

Appendix 3 – Cont.

UAM100040083	*Pardosa tristis* (Thorell, 1877) 1 female	COLORADO
UAM100040085	*Pardosa tristis* (Thorell, 1877) 1 female	COLORADO
UAM100040086	*Pardosa tristis* (Thorell, 1877) 1 male	COLORADO
UAM100040087	*Pardosa tristis* (Thorell, 1877) 1 male	COLORADO
UAM100040088	*Pardosa tristis* (Thorell, 1877) 1 male	COLORADO

Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402
Green Mountain Res. MacDonald Flats CG	39.84839	-106.23555	2402

Appendix 3 – Cont.

UAM100039724	*Pardosa tristis* (Thorell, 1877)	1 female	IDAHO
UAM100039725	*Pardosa tristis* (Thorell, 1877)	1 female	IDAHO
UAM100045694	*Pardosa tristis* (Thorell, 1877)	1 male	MONTANA
UAM100045715	*Pardosa groenlandica* (Thorell, 1872)	1 female	MONTANA
UAM100045716	*Pardosa groenlandica* (Thorell, 1872)	1 female	MONTANA
UAM100045719	*Pardosa groenlandica* (Thorell, 1872)	1 female	MONTANA
UAM100045720	*Pardosa groenlandica* (Thorell, 1872)	1 female	MONTANA

Snake River	43.72296	-112.08754	1447	
Snake River	43.72296	-112.08754	1447	A
Clark Canyon Res.	44.99837	-112.85577	1678	
Kalispel, Somers Beach	48.07571	-114.21905	883	
Kalispel, Somers Beach	48.07571	-114.21905	883	A
Kalispel, Somers Beach	48.07571	-114.21905	883	
Kalispel, Somers Beach	48.07571	-114.21905	883	

Appendix 3 – Cont.

RJ 1	*Pardosa tristis* 1 male (Thorell)	OREGON	Scroll Cart C.G.
RJ 2	*Pardosa tristis* 1 female (Thorell)	OREGON	Scroll Cart C.G.

45.15790	-118.37990	1554	A
45.15790	-118.37990	1554	